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Foreword to the Special Issue on the Experimental Lakes Area

This special issue of the *Journal* is devoted to 26 papers on the Experimental Lakes (ELA) of the Canadian Precambrian Shield located about 275 km east of Winnipeg, Manitoba. Documentation of these unique whole-lake experiments began with 14 background papers in a special issue of this *Journal* in 1971 (*J. Fish. Res. Board Can.* 28 (2)), and experimental approaches to limnology, which led to the many significant results reported herein, were explored in 13 papers published in 1973 (*J. Fish. Res. Board Can.* 30 (10)).

We are privileged to document and disseminate further results from this long-term research program, which stands out as one of the most productive interdisciplinary ventures in Canada. As Schindler indicates in the introduction to this issue, the scientific evidence generated from the ELA program provides the necessary justification for research managers at the Freshwater Institute for supporting interdisciplinary long-term research and for their determination to provide a healthy climate for such research. There has been an incalculable return on the investment in the research in terms of knowledge that can now be applied with confidence to solve specific management problems. The fact that so many of the original team are still together is indeed a remarkable testimony to the research climate afforded by the Freshwater Institute and the team leader, David Schindler.

This issue along with those published previously on ELA in the *Journal* will be standard reference material for limnologists for years to come. Reprints of specific papers will be available from each author. A limited number of the whole issue will be available from the Freshwater Institute.

J. WATSON
Acting Director and Editor

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Avant-propos au numéro spécial sur la région des lacs expérimentaux

Ce numéro spécial du *Journal* contient 26 articles traitant des lacs expérimentaux (ELA) du Bouclier précambrien canadien situés à quelque 275 km à l'est de Winnipeg, au Manitoba. Les résultats de ces expériences uniques sur lacs entiers parurent d'abord dans 14 exposés publiés dans un numéro spécial du *Journal* en 1971 (*J. Fish. Res. Board Can.* 28 (2)). L'approche expérimentale à la limnologie, qui engendra plusieurs des importants résultats décrits dans le présent numéro, fut examinée dans 13 articles publiés en 1973 (*J. Fish. Res. Board Can.* 30 (10)).

C'est un privilège de publier des résultats additionnels de ce programme de recherche à long terme, l'un des projets interdisciplinaires les plus productifs au Canada. Comme le mentionne Schindler dans l'introduction, les résultats scientifiques engendrés par le programme de la ELA militent en faveur de l'appui à donner par les gestionnaires de l'Institut des eaux douces à la recherche interdisciplinaire à long terme et de leur détermination d'assurer un climat sain pour une telle recherche. Les investissements eurent un rendement incalculable en termes de connaissances qui peuvent maintenant être appliquées avec confiance à la solution de problèmes spécifiques de gestion. L'équipe originelle est encore à peu près intacte : cela témoigne en vérité du climat de recherche que fournissent l'Institut des eaux douces et le chef de l'équipe, David Schindler.

Pour plusieurs années à venir, ce numéro et les numéros précédents du *Journal* consacrés à la ELA serviront référence aux limnologistes. Chaque auteur disposera de tirages à part d'articles particuliers. On pourra se procurer le numéro entier auprès de l'Institut des eaux douces, qui en aura un nombre limité.

J. WATSON
Directeur et rédacteur intérimaire

Evolution of the Experimental Lakes Project

D. W. SCHINDLER

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

Introduction

Although experimental manipulation of whole water bodies or large enclosures is not new, being practiced by fish culturists for centuries and by numerous experimental investigators for over 30 years, it is only in the last decade that the techniques have become firmly established as tools for investigating a wide variety of management problems in marine, estuarine, and fluvial environments as well as in lakes.

In 1968, the Government of Canada and the Province of Ontario agreed to the establishment of an Experimental Lake Area (ELA) in Northwestern Ontario (Johnson and Vallentyne 1971). The purpose of designating this area was to permit experimentation on a whole-lake scale to resolve questions related to the eutrophication issue. Data resulting from these experiments have proved useful in forming nutrient control strategies for larger lakes. However, the project has not confined itself to this single management issue but has become involved in a number of important ecological problems. Using whole-lakes, whole watersheds, or large enclosures within lakes, the effects of acidification, pollution with heavy metals or radionuclides, clearcut logging, nitrilotriacetic acid (NTA), ameliorating the effects of nutrient addition, and a natural windstorm and forest fire have been explored. This volume contains a cross-section of papers describing several of these studies, the long-term background work and process-oriented research that allowed data from the treated ecosystems to be interpreted, and methodological work that was necessary in the course of the experiments.

Long-Term Background Data

At times ELA has been criticized by some for placing too much emphasis on "pure" or "basic" research. In fact, such work has provided the key to success of management-oriented studies. Often the results of a subtle, deliberate perturbation can only be interpreted with confidence by understanding the natural variability inherent in unmodified lake systems. The papers in this volume illustrate the necessity for long-term control studies to compliment experimental manipulations.

For example, the eutrophication studies, the acidification of Lake 223, and the interpretation of the effects of forest fire all rely on the long-term hydrological, chemical, and limnological studies of the Rawson Lake watershed (Newbury and Beaty 1980; Schindler et al. 1976; Schindler et al. 1980b) and on in-depth analyses of data from control and experimentally modified ecosystems (for example, Fee 1979, 1980). Likewise, the study of long-term variation in control systems has been fundamental to assessing the utility and efficiency of hypolimnion injection of nutrients (Schindler et al. 1980c) and control of phosphorus sources as means of controlling eutrophication (Schindler 1974, 1975, 1977). As a rule of thumb, most commonly measured parameters will have annual means which vary by 2× or more from one year to another without any human-induced modifications. An exception is primary production (Fee 1980, Table 3), where 95% of all annual values lie within 30–40% of the mean. Such variations cannot be ignored in interpreting changes in lake systems.

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Methodology and Process-Oriented Research

Research into methodology and processes can be difficult to defend to the manager who wants stress placed upon immediate solutions to the problems facing him. However, such studies are very basic to the acquisition of data of the quality necessary for sound management decisions. Consequently the ELA project (and its associates) have made an effort to pursue such studies in the past (for example *J. Fish. Res. Board Can.* 30(10), 1973).

This volume contains some spectacular examples of how inadequate methodology can lead to erroneous conclusions. Stainton (1980) and Levine and Schindler (1980) show that the common molybdate method overestimates orthophosphate in Shield lakes by many fold. Graham et al. (1980) have shown that the commonly assumed factor of 3 for converting acetylene-reduction rates to nitrogen fixation rates can vary widely, causing improper assessment of this source of nitrogen income for lakes. Mills and Beamish (1980) show how errors associated with scale ageing can affect the interpretation of fish population dynamics, and offer a solution, at least for lake whitefish, by employing fin-ray sections.

Some of the process studies at ELA have vastly altered conceptual and mathematical models of the lake systems, as well as allowing sampling programs for the lakes to be streamlined efficiently.

For example, the diffusion studies of Quay et al. (1978) have shown that chemical concentrations below the thermocline are more likely to be the result of horizontal transport than vertical diffusion. Hesslein (1980a) has convincingly shown the utility of knowing this fact in his model of the distribution of sediment-derived chemical species. The fact that vertical diffusivities are very low means that the contribution of diffusion from the hypolimnion is an insignificant source of nutrients to the epilimnion; this vastly simplifies nutrient budgets for these regions (Schindler et al. 1977) and illustrates the utility of hypolimnion nutrient disposal (Schindler et al. 1980c). On the other hand, diffusion studies of sediment pore water (Hesslein 1980b) show that epilimnion sediments may be an important source of nutrients for surface waters (Schindler et al. 1977).

The studies of Bower and McCorkle (1980), Hesslein et al. (1980a), and Fee (1980) have greatly increased our knowledge of primary production and related processes. It is clear that gas exchange with the atmosphere is a major process affecting the dissolved inorganic carbon (DIC) pool in an oligotrophic lake like Lake 224. The low specific activity of the dissoved organic carbon (DOC) pool appears to indicate that it is derived to a large extent from allochthonous sources, and does not participate to a significant degree in the energy budget of these lakes, at least on the short-term.

Injection of ^{14}C directly into a lake or large enclosure should, of course, give the best possible assessment of primary production. Comparison of in situ uptake with results from Fee's incubator indicates that the latter, as used at ELA, gives reasonably reliable results.

Fee's (1980) overview of all ELA incubator results leads to a number of surprising conclusions. His bold suggestion that surface solar insolation measurements may not be needed due to the good correlation ($r = 0.997$, $n = 53$) between annual production in "actual" and "cloudless" weather is surprising, but if shown to be widespread, would spare small projects the expense of making such measurements. Likewise, the tight relationship between "column" and "morphometry corrected" production may be a considerable time and work saver, if it proves to hold for areas outside ELA. Other proven departures from standard practice which are shown to be considerable time savers are the use of integrated samples to characterize the epilimnion and deeper strata and the illustration that diurnal variation is of little significance when estimating long-term production rates. On the other

hand, light quality was shown to be of great significance when measuring production in subthermocline layers.

Chan and Campbell (1980) demonstrate that even in the very eutrophic Lake 227, denitrification is an insignificant component of the nitrogen budget of the lake. On the other hand, Flett et al. (1980) demonstrate that fixation of atmospheric nitrogen can be a major source of this element for lakes when the ratio of N/P from other inputs is low. As a result, lakes in the area are seldom, if ever, nitrogen limited, as Healey and Hendzel (1980) show, and the standing crop of phytoplankton is proportional to phosphorus input, corrected for water renewal (Schindler et al. 1978).

Turning to heavy metal and radionuclide experiments, Jackson et al. (1980) show that when metals are sedimented, most are bound to the NaOH extractable (presumably humic acid) fraction of the sediments.

Results of Significance for Management

Our previous publications (for example, Schindler 1974, 1975, 1977; Schindler et al. 1978), plus the works of many others, have shown the importance of phosphorus control in managing eutrophication. Yet controlling all sources of phosphorus is not always possible. Injection of effluents into the hypolimnion (Schindler et al. 1980c) appears to be one way that the eutrophying effect of phosphorus can be slowed considerably or possibly even reduced in total impact.

Davies (1980) has shown that increased nutrient input and production leads to a corresponding increase in emerging insects, implying that growth of benthos-feeding fishes, too, should increase. Thus, moderate eutrophication has positive as well as negative effects.

A number of useful results have already come from the acidification of Lake 223 (Schindler et al. 1980d). Sulfuric acid appears to have less than 50% of the expected capacity for depleting dissolved alkalinity. Additional bicarbonate buffering is supplied by the concentration of DIC respired in the course of microbial reduction of sulfate to sulfide in the hypolimnion and the mobilization of ferrous iron from sediments. This source of protection against acidification has not previously been recognized. Several heavy metals, thought to originate from atmospheric fallout or from terrestrial soils in the past, have been shown to originate from lake sediments as well (Schindler et al. 1980a). Neither of these studies could have been satisfactorily done in lakes in areas of high acid fallout. Malley (1980) has shown that at pH <6, moulting ability of crayfish is severely impaired. These are a numerous and important food organism for fishes in lakes of the area, and any significant decline in abundance would undoubtedly have serious consequences. Müller (1980) has shown that the species diversity and numbers of periphyton decrease under acid conditions, and that the dominant group becomes Chlorophyta instead of Diatomeae.

Hesslein et al. (1980b) show that even though the removal of several metals and radioisotopes from the water column occurs at somewhat similar rates, the pathways for removal may be quite different. Metals which remain in solution, such as selenium and cesium, appear to adsorb to epilimnion sediments, and are slow to reach the deeper regions of the lake. On the other hand, substantial percentages of cobalt and iron, which quickly adsorb to seston, reach the hypolimnion by sedimentation of particulate matter. These differences in distribution and pathway will greatly influence the components of the food chain which will be exposed to a given metal, as well as the mechanism of uptake (Hesslein et al. unpublished data).

In field toxicology experiments, Marshall and Mellinger (1980) and de Noyelles et al. (1980) show that even a few parts per trillion of cadmium may have devastating effects on zooplankton and phytoplankton populations. These

studies differ from commonly used laboratory toxicology studies in that they employ natural species assemblages in natural environments, and the duration of exposure is greater than a generation time for many of the organisms. A disadvantage of such systems is the fact that it is impossible to separate direct toxicity of cadmium from secondary effects. For example, at 1 $\mu g\,Cd \cdot L^{-1}$, Marshall and Mellinger (1980) found an increase in rotifers. Yet at 3 $\mu g \cdot L^{-1}$, rotifer populations were severely reduced. It is conceivable that the increase at 1 $\mu g \cdot L^{-1}$ is due to a release from competition for food by nauplii, which are reduced at 1 $\mu g \cdot L^{-1}$, while rotifers were able to tolerate this concentration without effect.

Extending such studies to even more detail, Knoechel and de Noyelles (1980) were able to detect the effects of phosphorus and light on the photosynthesis of individual algal species. Such techniques applied to toxicological problems allow predictions to be made at the species level.

While whole-lake experiments provide valuable insights, they cannot be done in most areas, due to the difficulty of obtaining exclusive use of water bodies for long periods. Therefore we have also used large enclosures within lakes to overcome the deficiencies of small scale laboratory experiments. Such techniques using 1 m diameter tubes, were first used in 1969 (Schindler et al. 1971). Batteries of such tubes were subsequently used for tests on NTA in 1971, and for numerous problems after that. Larger "limnocorrals" were used in gas exchange and mixing studies in 1972 (Hesslein and Quay 1973). Ten-metre diameter tubes were first used in acidification studies in 1976 (Schindler et al. 1980a).

Brunskill et al. (1980) used large in situ enclosures to study chemical and biological processes which affect the oxidation and reduction of arsenic. This work provides valuable background for management regulations governing the disposal of arsenic, a problem causing much concern in the Winnipeg and Yellowknife areas.

Bower and McCorkle (1980) studied the uptake of radiocarbon by algae in large enclosures. Marshall and Mellinger (1980) and Müller (1980) have used them in ecosystem-scale studies of the toxicity of pollutants to zooplankton and periphyton. The use of enclosures of several sizes and types will continue to be used at ELA to discern how such mesoscale experiments duplicate the changes that occur when a whole ecosystem is perturbed.

Schindler et al. (1980b) have demonstrated that while detectable increases in runoff of nutrients are caused by natural windstorms and forest fires, amounts of nutrients lost are too small either to deplete the fertility of forest soils or to cause eutrophication problems in receiving lakes. Chemical data for before and after such disasters have not previously been available, and such findings should have some influence on the development of fire suppression policies by forest managers.

The Influence of Personnel at ELA

Much of the success of the ELA project must be attributed to the long-term dedication of many people. Of the contributors to the 1973 volume, almost all are still with the project. Several key scientists and senior technical staff have been with the project for 10–12 years, with enthusiasm still undampened by boredom or by the lure of more lucrative and prestigious positions. One finds that insights are sharpened, consistency of results is maintained, and multidisciplinary studies are much more easily planned by staff who have developed mutual interests and trust by years of working together. The enthusiastic participation of geochemists from Lamont–Doherty Geological Observatory, which has been continuous since 1969, has also been a major factor in the success of many experiments. Visitors from several other institutions have also played key roles at critical times.

ELA was fortunate to attract an unusually keen group of graduate students from several institutions early in its development. Many of these have proved to be

amazingly productive, contributing substantially to this, as well as the previous volume.

Finally, the working conditions which make such an environment possible to maintain are in turn attributable to long-term interest of several long-suffering managers at the Freshwater Institute, who have often supported the project's pursuit of long-term management problems, even when under heavy pressure to divert resources to more politically expedient short-term investigations. In historical order, these include W. E. Johnson, J. R. Vallentyne, S. E. Schick, A. L. Hamilton, G. H. Lawler, R. D. Hamilton, and their staff. Mr Willy Burton, and Cliff Jones and his staff in the FWI shops have bailed us out of many a crisis over the years. Mr Shelly Zettler and his staff in graphics have supplied most of the drawings for this, as well as previous volumes.

Last, but not least, Drs Kaz Patalas, R. D. Hamilton, and Peter Dillon criticized all of the papers in this volume, some in several drafts. Many improvements resulted from their suggestions. We hope that the contents of this volume will illustrate the important position which large-scale ecosystem experiments occupy as intermediate between laboratory studies and ecosystem management.

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Hypolimnion Injection of Nutrient Effluents as a Method for Reducing Eutrophication

D. W. SCHINDLER, T. RUSZCZYNSKI, AND E. J. FEE

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

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Injection of nutrients into the anoxic hypolimnion of a small Precambrian Shield lake for 5 yr caused less of a eutrophication problem than discharging nutrients into surface waters. Phytoplankton standing crop and production in the whole lake averaged only 10–21% of values in a nearby lake fertilized at the surface. Five-year averages for the epilimnion only were still lower: 5–8% of those in the surface-fertilized lake. Analysis of long-term trends in chlorophyll and nutrient concentrations revealed much slower rates of increase than in surface-fertilized lakes.

Key words: eutrophication, hypolimnion injection, sediment–water interaction, nutrients, experimental lakes

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L'injection d'éléments nutritifs dans l'hypolimnion anoxique d'un petit lac du Bouclier précambrien durant 5 ans a causé un problème d'eutrophisation moins sérieux que la décharge d'éléments nutritifs dans les eaux superficielles. La biomasse et production de phytoplancton dans tout le lac ne sont en moyenne que de 10 à 21% des valeurs observées dans un lac avoisinant fertilisé à la surface. Les moyennes de 5 ans pour l'épilimnion seul sont encore plus basses: 5 à 8% de celles du lac fertilisé à la surface. L'analyse des tendances à long terme des concentrations de chlorophylle et d'éléments nutritifs révèle des taux de croissance beaucoup plus lents que dans les lacs fertilisés à la surface.

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EUTROPHICATION control schemes have focused almost entirely on reducing phosphorus input (Vollenweider 1968, 1976; Anon. 1969; Dillon and Rigler 1975; Schindler et al. 1978). This has proven effective in lakes where sources are discrete and easily controllable (Edmondson 1970; Michalski et al. 1975; Ahlgren 1972, 1977; Schindler 1975; Larsen et al. 1975), but there are many lakes where such measures are too costly. This may be the case in the St. Lawrence Great Lakes. While the 1972 Water Quality Agreement between the USA and Canada states that phosphorus concentrations at all sewage treatment plants influent to the Great Lakes should be reduced to 1 mg/L before discharge, only 28% of the treatment plans surveyed in 1976 met this standard (International Joint Commission 1976). More recent investigations of phosphorus loading to Lakes Erie and Ontario reveal that an even lower concentration in discharge would be necessary for substantial recoveries of these two lakes (Anon. 1978). Furthermore, population and industry in the Great Lakes basin are increasing continuously, causing increased quantities of wastes to be discharged to the lakes. It thus appears that increasing cultural pressures, cost and the long time-lag inherent in implementing nutrient control schemes will all hinder the recovery of the Great Lakes from eutrophication. Similar conclusions may be drawn for other lakes in populous areas of the world. As a result, it is necessary in many cases to look for alternatives or supplements to eliminating nutrient sources to assist in reducing the eutrophication problem.

Several alternatives or supplements to elimination of nutrient sources have been tried in the past. A number of investigators have used siphons to discharge nutrient-rich waters from the hypolimnions of small lakes (for example, Olszewski 1961; Gächter 1976; Pechlaner 1975). Others have attempted to stop phosphorus release from sediments by aerating the hypolimnion (Bernhardt and Wilhelm 1975; Ohle 1975) or by using the same technique to keep thermal stratification from developing (Dunst et al. 1974). Artificial manipulation of the food chain to enhance grazing of phytoplankton has also been tried (Shapiro et al. 1975). None of the above schemes could be simply employed in a large lake system at the present time.

While performing whole-lake fertilization and mixing experiments, we obtained results which suggested that the eutrophying effect of nutrients might be reduced by injection of nutrients into the hypolimnion

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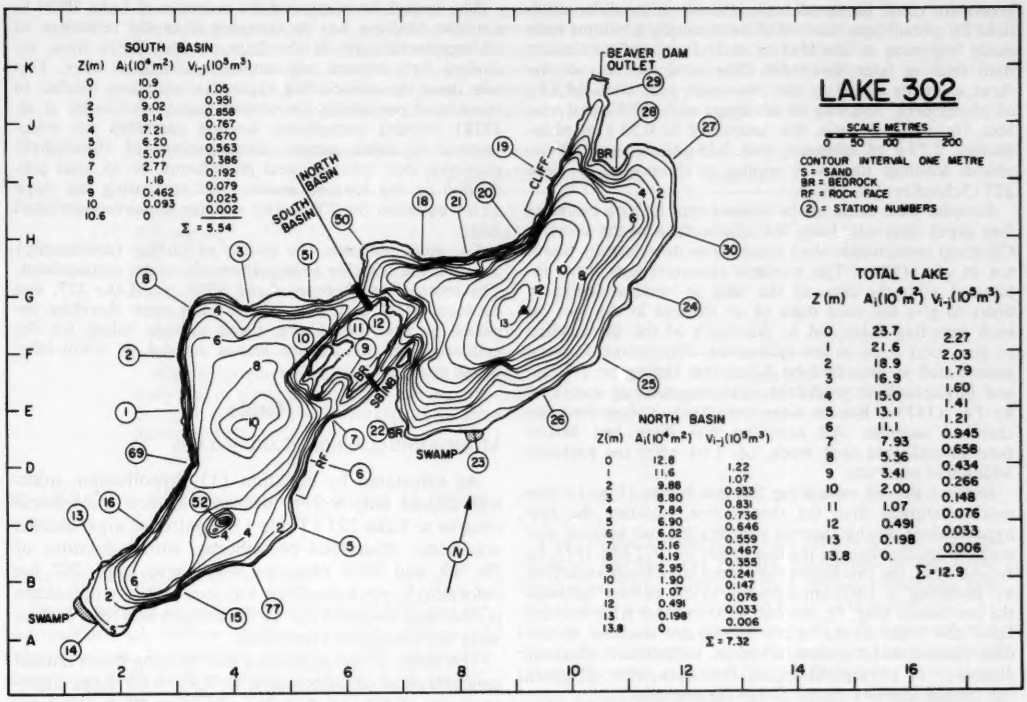

FIG. 1. A morphometric map of Lake 302 (slightly changed from Brunskill and Schindler (1971)). The position of the curtain is shown by a heavy bar between the basins. The point of nutrient addition is shown by (▲).

rather than discharging them into surface waters as is commonly done. Firstly, we observed that phosphorus added to the lakes as phosphate was efficiently transferred to sediments, with little or no return, even under anoxic conditions (Schindler et al. 1977). The reason for this was that phosphate added to the hypolimnion was quickly taken up by seston (presumably algae and bacteria) and therefore rendered susceptible to sedimentation (Levine 1975; Levine and Schindler 1980). Once in sediments, phosphorus was efficiently bound to colloidal matter, so that there was no detectable return to overlying water (Jackson and Schindler 1975; D. R. S. Lean and D. W. Schindler unpublished experiments). Secondly, vertical mixing experiments in small lakes showed that vertical mixing was extremely low so that no significant return of nutrients would be expected from the hypolimnion, even when nutrient gradients were large.

Based on these results, we hypothesized that injection of nutrients into the hypolimnion, below the euphotic zone, might allow most of the phosphorus to be permanently transferred to sediments before it could reach surface waters to cause algal blooms. We devised a whole-lake experiment to test this hypothesis.

Description of the Lake

Lake 302 has two basins of roughly equal size separated by a narrows, which is both shallow and partially blocked by an island (Fig. 1). The lake is typical of small lakes in the Experimental Lakes Area. Its protected nature prevents the lake from undergoing complete overturn in spring, so that the hypolimnion is devoid of oxygen for several months of the year, even in the year before experimental fertilization was begun. Fall overturn was complete in all of the years studied.

Materials and Methods

Nutrient additions were made using a 200-L barrel mounted on a raft anchored near the center of the North Basin, at the deepest point in the lake. Fertilizer was placed in the barrel and dissolved in water pumped from a depth of 8 m, which was well below the thermocline. The outlet of the barrel, draining the dissolved nutrients back to the hypolimnion, was 1 m deeper than the inlet and on the opposite side of the raft. Screens in the barrel kept undissolved fertilizer from being washed into the hypolimnion. Each weekly addition consisted of 7.5 L of 85% phosphoric acid, 68.2 kg of NH_4Cl, and 56.8 kg of sucrose. Reduced forms of nitrogen and carbon were used to maintain the hypolimnion of the lake in an anoxic condition, so that the

procedure could be tested under the worst possible conditions for phosphorus removal. Fifteen weekly additions were made beginning in late May or early June, and continuing until mid- to later September. The total amounts of the three elements added to the lake each year were 68.5 kg of phosphorus, 356.9 kg of nitrogen, and 477.1 kg of carbon. On an areal basis, this amounted to 0.54 g of phosphorus, 2.79 g of nitrogen, and 3.73 g $C \cdot m^{-2} \cdot yr^{1-}$. Phosphorus loadings were very similar to those used in Lake 227 (Schindler et al. 1973).

Samples were taken at the deepest spot in both basins at 1-m depth intervals, from the lake surface to the bottom. Chemical constituents were analyzed as described by Stainton et al. (1974). The nutrient concentrations were integrated with the area of the lake at various depths in order to give the total mass of an element in the lake on each sampling date, and to the depth of the thermocline to give total mass in the epilimnion. Phytoplankton were enumerated as described by Kling and Holmgren (1972), and phytoplankton production was measured as described by Fee (1973). Results were integrated as described for chemical samples. All sampling was done just before nutrient additions each week, i.e. 1 wk after the previous addition of nutrients.

Because the sill separating the two basins (Fig. 1) was much shallower than the thermocline, isolating the two hypolimnions during thermal stratification, no attempt was made to totally separate the two basins in 1972 and 1973. In July of 1974, the two basins were more completely separated by installing a vinyl-impregnated nylon curtain between the two basins (Fig. 1), in order to keep fishes from moving from one basin to the other. Subsequent analyses of the data showed that the curtain had an insignificant effect on chemistry or phytoplankton, so that data from all years are treated together in the following analysis.

Analysis of the Experiment

The effectiveness of hypolimnetic disposal was evaluated in two ways. Firstly, the amounts of chlorophyll, phytoplankton volume, phytoplankton production, total phosphorus, total nitrogen, and suspended carbon plus dissolved inorganic carbon in Lake 302N were compared with the control basin, Lake 302S, and with Lake 227, which received nearly the same phosphorus loading, added to surface waters. To compare hypolimnetic and surface additions, the following formula was used to calculate an efficiency factor (E):

$$(1)\quad E = 100\left(1 - \frac{\bar{C}_{302N} - \bar{C}_{302S}}{\bar{C}_{227} - \bar{C}_{302S}(F_p)}\right)$$

where $\bar{C}$ is the mean annual concentration of each parameter (except for production, where total production in the ice-free season is used), and F_p is the ratio of loading with phosphorus, corrected for water renewal.

$$(2)\quad F_p = \frac{I_p V_o^{-1} 302N}{I_p V_o^{-1} 227}$$ when I_p is the mean annual input

of phosphorus and V_o is the mean annual volume of outflow (Schindler et al. 1978). $I_p V_o^{-1}$ values used were for 1972–76 averages for 302N and 1970–74 averages for 227. F_p averaged 1.15. From the table in Brunskill and Schindler (1971), water renewal rates and outflow volumes for lakes 227 and 302 (both basins) are much lower than those used here, which are measured values.

The second assessment of the response of Lake 302N to nutrient addition was to compare it to the responses of all monitored lakes in the Experimental Lakes Area, including both control and artificially fertilized ones. This was done by constructing regression equations similar to those used previously for surface inputs (Schindler et al. 1978) relating phosphorus loading corrected for water renewal to mean annual concentrations of chlorophyll, phytoplankton volume, total phosphorus, or to total production in the ice-free season, and comparing the slope of the equation for 302N with that for all surface-enriched lakes.

For most purposes, the quality of surface (epilimnetic) waters is the criterion of importance for water management. The comparisons between Lake 302N and Lake 227, and between 302N and other ELA lakes were therefore repeated as described above, using average values for the epilimnion in the ice-free season instead of whole-lake, whole year data.

Results

Mean Annual Whole-Lake Values

As calculated by equation (1), hypolimnion injection caused only a 27% decrease in total phosphorus relative to Lake 227 (Table 1). Control of algal blooms was better than total phosphorus, with reductions of 79, 80, and 90% observed relative to Lake 227 for chlorophyll, phytoplankton volume, and phytoplankton production, respectively. Total nitrogen and total carbon were also efficiently controlled.

The slope of the regression line relating mean annual concentration of chlorophyll to $I_p V_o^{-1}$ for Lake 302N was only 5.2% of that for all other ELA lakes receiving nutrients at the surface (Fig. 2). Efficiencies calculated similarly for total phophorus and phytoplankton volume were 10.0 and 8.5%, respectively. By expressing these in a similar fashion to the E values calculated above, one concludes that hypolimnetic injection caused a 95% reduction in chlorophyll, a 90% reduction in total phosphorus, and a 92% reduction in phytoplankton volume.

Summer Epilimnion Values

Algae and nutrients in the epilimnion of Lake 302N were even less affected by nutrient additions than those in the whole lake. When compared to lakes 227 and 302S by equation (1), E values were 95, 93, and 92% for chlorophyll a, phytoplankton volume, and phytoplankton production, respectively (Table 2). E values for ΣP, ΣN, and ΣC were 83, 88, and 94%, respectively.

When linear regression was used to compare epilimnion values in Lake 302N to the epilimnions of surface-loaded lakes, similar conclusions resulted. The ratio of slopes showed an efficiency of 99% for total phosphorus, 100% for chlorophyll, 98% for phytoplankton volume, and 97% for primary production.

All of the above values are within the limits of error associated with measuring nutrients or phytoplankton, so that one might conclude that hypolimnion injection

TABLE 1. A comparison of whole-lake mean annual concentrations of nutrients, chlorophyll, phytoplankton volume and production in a control lake (Lake 302S), and two lakes receiving roughly equal nutrient loading in the hypolimnion (Lake 302N) and the epilimnion (Lake 227). Data are 5-yr averages, except for phytoplankton production, where only data for 4 yr were available for Lake 302. *E* is the calculated efficiency of hypolimnion injection at reducing concentration or production relative to surface additions, as calculated by equations (1) and (2) in text.

	Lake 302S	Lake 302N	Lake 227	*E*, %
$\Sigma\bar{P}$, mg·m^{-3}	10.2	31.0	34.9	27
$\Sigma\bar{N}$, mg·m^{-3}	340.0	556.0	1035.0	73
$\Sigma\bar{C}$, mg·m^{-3}	2401.0	2808.0	7071.0	92
Chlorophyll *a*, mg·m^{-3}	5.3	11.3	30.4[a]	79
Phytoplankton volume, mm^3·m^{-3}	1514.0	2576.0	6107.0	80
Phytoplankton production, ice-free season, g C·m^{-2}·yr^{-1}	48.0	59.8	154.3	90

[a]One chlorophyll value for Lake 227, in 1967, 18 μg·L^{-1} (Patalas 1971) appears to be an analytical or typographical error and was omitted. All other prefertilization data for Lake 227 (for 1968 and early 1969) were typical for small lakes in the area (2–5 μg·L^{-1}) (Armstrong and Schindler 1971).

caused no detectable change in the quality of surface waters.

OTHER INDICES OF EUTROPHICATION

The magnitude of maximum surface blooms and the transparency of surface waters are other indices of eutrophication. It therefore appears worth comparing these as well. Regardless of which parameter is picked,

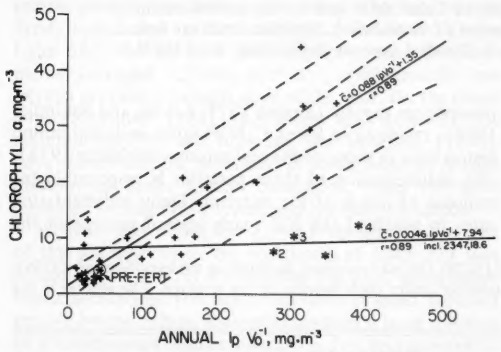

FIG. 2. Regressions relating mean annual chlorophyll and phosphorus loading corrected for water renewal ($I_pV_o^{-1}$) for surface-fertilized lakes at ELA and for Lake 302N, fertilized in the hypolimnion. The ratio of the slopes is 0.052. Efficiencies for total phosphorus and phytoplankton volume were calculated similarly, as described in the text. The coordinates (2347, 18.6) are for the year 5 points, in an extremely dry year. Surface-fertilized lakes include those receiving only natural sources, and are designated by (+). Lake 302N is designated by (*). Each point represents one lake-year. The numbers represent the years of loading (year 1 = 1972).

conclusions are identical to those reached above (Table 3).

Due to the strongly reduced nature of the injected nutrient, oxygen was rapidly consumed in the hypolimnion of Lake 302N, so that anoxic conditions prevailed there for an average of 8 mo of the year (Fig. 3). Despite this, strong net removal of added phosphorus, nitrogen, and carbon from the water column occurred throughout the period (Schindler 1975).

No significant differences in species composition of phytoplankton in the two basins were observed during the experiment. Chrysophyceae dominated the epilimnions of both basins throughout all seasons of the year. A very small bloom of *Anabaena planctonica*, constituting 17 and 10% of the total biomass in the North and South basins, respectively, occurred in early September of 1976. This is not an unusual occurrence for natural lakes of the area. Both basins exhibited subepilimnion peaks of Chrysophyceae in summer. These were usually larger in the North Basin due to the increased concentrations of nutrients. Fee (1976) gives more detailed information on subepilimnion blooms, and Findlay (1978) gives a complete description of phytoplankton species and biomass in the two basins.

Discussion

There are a number of reasons why hypolimnetic injection of nutrients was effective in Lake 302. During thermal stratification, the thermocline is an extremely effective barrier to passage of dissolved substances. In a series of tritium injection experiments, Quay et al. (1980), found that all vertical mixing processes together, including internal seiche activity, could be accounted for by a vertical eddy diffusion coefficient of less than 10^{-4} cm^2·s^{-1} in lakes the size of 302N, representing a passage of less than 10 μg P·m^{-2}·d^{-1} from

TABLE 2. A comparison of concentrations of nutrients, chlorophyll, phytoplankton volume and production in the epilimnion during the ice-free season. Data are 5-yr averages, except for phytoplankton production, where only data for 3 yr were available. Lakes and calculations are as in Table 1.

	Lake 302S	Lake 302N	Lake 227	E, %
$\Sigma\bar{P}$, mg·m^{-3}	7.6	13.8	39.8	83
$\Sigma\bar{N}$, mg·m^{-3}	283.0	358.0	833.0	88
$\Sigma\bar{C}$, mg·m^{-3}	1649.0	1878.0	5389.0	94
Chlorophyll *a*, mg·m^{-3}	4.0	6.1	38.8	95
Phytoplankton volume, mm^3·m^{-3}	1797.0	2394.0	9499.0	93
Phytoplankton production, g C·m^{-2}·yr^{-1}	25.7	39.3	172.1	92

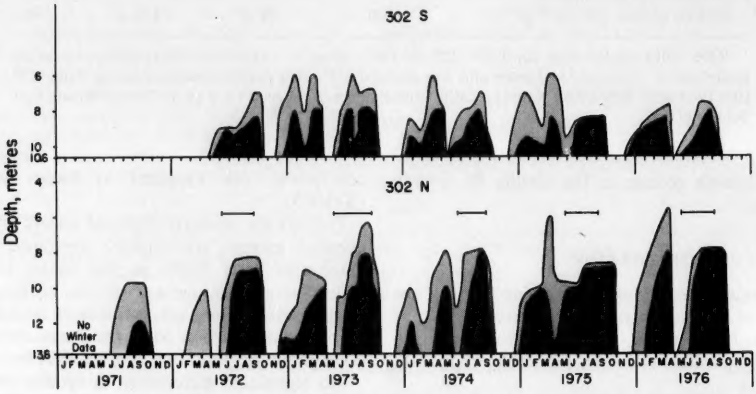

FIG. 3. Oxygen conditions in the hypolimnions of Lake 302N and in the control basin, Lake 302S. The horizontal bars indicate the period of fertilization. Stippled areas are less than 2 mg·L^{-1}, dark areas less than 0.1 mg·L^{-1}. The first year of fertilization for L302N was 1972.

the hypolimnion into the epilimnion of the lake at the maximum phosphorus gradients observed. In an entire growing season, this would be less than 1% of the external phosphorus supply to a eutrophic lake loaded at the same rate.

Hypolimnetic algae and bacteria are able to take up phosphorus rapidly (Levine 1975; Levine and Schindler 1980), resulting in lower C:N:P ratios in hypolimnion seston than in those of the epilimnion (Schindler 1976). The sedimentation of these particles is responsible for removal of much of the nutrient. Daily sedimentation rates in nearby Lake 227 reach several percent of the

TABLE 3. A summary of water quality indices for the control lake (302S), the lake receiving fertilizer in the hypolimnion (302N), and the lake receiving fertilizer in surface water (227). The first number under each heading is the average. The second is the "worst case" recorded in each of 5 different yr.

	Secchi depth (m)		Chlorophyll mg·m^{-3}		Phytoplankton volume mm^3·m^{-3}		Extinction coefficient		ΣP, mg·m^{-3}	
Whole lake, annual										
302S	3.63	2.75	5.3	11.7	1514	4 777	0.70	0.91	10	30
302N	3.23	1.75	11.3	36.2	2020	11 751	0.74	1.08	31	90
227	1.52	0.40	30.4	152.0	6107	37 917	1.49	2.94	35	108
Epilimnion, ice-free season										
302S	—		3.8	9.4	1797	4 777	—		7	36
302N	—		6.1	16.5	2394	11 751	—		14	49
227	—		38.8	160.6	9499	37 917	—		40	103

mass of total phosphorus in the lake each day in late summer (Schindler et al. 1973).

Finally, our laboratory and in situ column experiments have shown that even dissolved phosphorus is efficiently and permanently removed by sediments, both in the presence and absence of oxygen. Thus several mechanisms, including chemical, physical, and biological ones, appear to be responsible for the success of hypolimnetic injection.

The results of this experiment may also give some insight into the role of deepwater sediments in hindering the recovery of lakes once external phosphorus sources have been reduced. If one assumes that our hypolimnetic injection is equivalent to return of nutrients from hypolimnetic sediments, our rate of addition would be equal to a phosphorus release rate of 778 $mg \cdot m^{-2} \cdot d^{-1}$ from sediments below the thermocline of Lake 302, comparable to rates which Ahlgren (1977) recorded from hypereutrophic Lake Norrviken. It appears that even if a large quantity of phosphorus is regenerated from deep sediments of eutrophic lakes, it should have much less effect than an equal amount added to surface waters, even if vertical mixing is two orders of magnitude higher than in Lake 302. Ahlgren's observations that after nutrient diversion the phosphorus concentration in Lake Norrviken decreased in proportion to the surface loading and water renewal rate, despite high rates of regeneration from sediments, is consistent with this observation.

The fact that net movement of phosphorus was always into Lake 302 sediments warrants special discussion. The belief that all sediments in all lakes must release phosphorus under anoxic conditions has become firmly entrenched in the limnological literature. Yet in Lake 302, the net flux of phosphorus *into* the sediments averaged 1.28 $mg \cdot m^{-2} \cdot d^{-1}$, even though low oxygen prevailed through most of the year. On the other hand, Mortimer (1941–42), Burns and Ross (1972), Ahlgren (1977), and Imboden and Emerson (1978) have shown that the sediments of some lakes do release phosphorus under anoxic conditions. Thus the sediments of all lakes do not respond similarly to anoxia.

Jackson and Schindler (1975) showed that much of the phosphorus in the sediments of Lake 227 was bound to the NaOH extractable fraction, presumable humic substances. It is likely that lakes in small, forested granite basins, where sediments contain a large amount of allochthonous organic debris but few redox-sensitive minerals, will react similarly. Whether sediments will become saturated after several decades of nutrient enrichment is not known, but after nine years of enrichment, no phosphorus is yet released from the anoxic hypolimnion sediments of Lake 227 (Schindler et al. 1977, and unpublished data).

Possible Deficiences of Hypolimnion Injection

While hypolimnetic injection works extremely well in Lake 302, a number of reservations must be stated about its long-term application to other lakes, particularly large ones. These have been pointed out by the many reviewers of this paper, and must be considered before applying the technique to a particular situation, therefore they seem worthy of mention here.

Some increase in the average total phosphorus and chlorophyll concentrations of Lake 302 is noticeable over the years. Because the period 1974–76 was unusually dry, $I_p V_o^{-1}$ was very high, and the observed increases appeared to be due to lower water renewal rates. Long-term (5-yr) trends average 8.0 mg $P \cdot m^{-3} \cdot yr^{-1}$ for the whole lake and 1.0 mg chlorophyll $a \cdot m^{-3} \cdot yr^{-1}$ for the epilimnion. It is however, also possible that the increases are due to a slow, but continuous enrichment of the lake which is unrelated to water renewal. If so, rates of increase are still many times slower than those observed in surface-fertilized lakes (D. W. Schindler unpublished data), and the method should still be useful as a long-term delaying tactic for postponing the effects of eutrophication. It is also impossible to predict whether equilibrium concentrations, once reached, will be lower due to hypolimnion injection than in surface-fertilized lakes. Several years of further study will be required to resolve this point.

Different responses may result in lakes where the exchange of phosphorus with sediments is controlled by the formation of redox-sensitive minerals rather than by reaction with organic matter, as discussed above. However, injection of well-oxidized effluent into a large, well oxygenated hypolimnion in such a lake might be expected to enhance the chemical removal of phosphorus by precipitation, so that even higher efficiencies than those observed in Lake 302N may result.

Effluent discharges are usually warmer, but more chemically concentrated than hypolimnion water, so that discharges may not remain at the depth of discharge. Gächter (1971) found that effluents discharged to Swiss lakes sank due to their high chemical density. This would tend to enhance the isolation of nutrients from the euphotic zone of the lake.

In a management situation, the nature of injected effluents may further complicate geochemical removal processes. For example, treatment of sewage for phosphorus removal using iron compounds may cause injection of effluents rich in iron, which could enhance the removal of phosphorus under oxic conditions. On the other hand, the use of alum for phosphorus removal often enriches effluents with sulfate, which might cause more iron to be precipitated as sulfides under anoxic conditions reducing the effectiveness of geochemical control. Thus, both the geochemical characteristics of a particular water body and the nature of an effluent may affect the results of a hypolimnetic disposal scheme.

In shallower lakes, where thermocline erosion may cause periodic entrainment of phosphorus into the euphotic zone throughout the summer (Burns and Ross 1972; Schindler and Comita 1972), hypolimnetic injection does not appear to be a viable management strategy.

The vertical transfer of substances in the thermocline of very large lakes may be up to 100× higher than in Lake 302, due to the much greater turbulence generated by wind in large systems (P. D. Quay, University of Washington, Seattle, Wash., personal communication). Even so, it would often be possible to inject nutrients at a greater distance below the thermocline than in Lake 302, and due to the large volume of the hypolimnion in such a lake, concentration gradients of phosphorus should be lower.

In very large lakes, zones of upwelling of hypolimnion water would probably have to be avoided when effluent pipes were installed. Likewise, installations would have to be designed to inject the effluents far enough from shore to be unaffected by the early-season thermal bar. Despite years of detailed study, our knowledge of the hydrodynamics of large lakes is still not complete enough to evaluate what effect large-scale mixing phenomena might have on a hypolimnion injection scheme, and cautious pilot studies of the technique should be done before it could be applied on a broad scale.

Altogether, we believe that hypolimnetic injection of point nutrient sources may be a useful supplement to source reduction as a means of reducing or considerably delaying eutrophication.

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Effects of a Windstorm and Forest Fire on Chemical Losses from Forested Watersheds and on the Quality of Receiving Streams

D. W. SCHINDLER, R. W. NEWBURY, K. G. BEATY, J. PROKOPOWICH, T. RUSZCZYNSKI, AND J. A. DALTON

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

SCHINDLER, D. W., R. W. NEWBURY, K. G. BEATY, J. PROKOPOWICH, T. RUSZCZYNSKI, AND J. A. DALTON. 1980. Effects of a windstorm and forest fire on chemical losses from forested watersheds and on the quality of receiving streams. Can. J. Fish. Aquat. Sci. 37: 328–334.

A severe natural windstorm followed by a high intensity forest fire caused significant increases in runoff and in losses of nitrogen, phosphorus, and potassium from two small Precambrian watersheds. Both the windstorm and the fire had significant effects on water and chemical yields. Water yields in the two basins were 1.6 and 1.8 times the pre-impact means, respectively, in the year after the burn. Maximum chemical losses were observed for nitrate, with values of 3.4 and 9 times the pre-impact means for the two basins in the year after the burn. Increases in annual yields of most chemical parameters were 1.1 to 2.9 times the background. Both increased concentrations and increased flow volumes appear to be responsible for the increased nutrient losses.

Key words: forest fire, windstorm, stream water quality, phosphorus, nitrogen, potassium losses, water yield

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Une forte tempête de vent suivie d'un intense feu de forêt eut pour effet une augmentation importante du ruissellement et des pertes d'azote, de phosphore et de potassium dans deux petits bassins hydrographiques précambriens. Le vent et l'incendie eurent tous deux d'importants effets sur les rendements en eau et en substances chimiques. Durant l'année qui suivit l'incendie, les rendements en eau dans les deux bassins furent 1,6 et 1,8 respectivement plus élevés que les moyennes d'avant ces catastrophes. C'est avec les nitrates qu'on a observé les plus grandes pertes de substances chimiques, les valeurs étant 3,4 et 9 fois plus grandes dans les deux bassins pendant l'année qui suivit l'incendie. Les augmentations de rendements annuels de la plupart des paramètres chimiques furent de 1,1 à 2,9 fois celles des concentrations de base. Ces concentrations et débits accrus semblent tous deux responsables de l'augmentation des pertes de substances nutritives.

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A number of recent publications have shown that the success of several important species of trees in boreal forests is dependent upon periodic forest fires (Wright and Heinselman 1973). Some investigators predict that current fire suppression policies will lead to the decline and possible extinction in the future of a number of important species and suggest that where human life and property are not endangered, forest fires should be allowed to burn or controlled burning should be undertaken. On the other hand, the general public has vigorously supported fire suppression, largely on esthetic or short-term economic grounds.

One poorly known factor in this controversy on forest management is the amount of nutrient lost from a watershed after it has been burned by a forest fire, and the effect of the nutrient on receiving waters. Several earlier studies of this subject (Wright 1976; McColl and Grigal 1975, 1977) have relied on the analysis of burned vs. control basins. However, in our experience, control streams which are side by side often have quite different water chemistry, even though superficially both watersheds look very similar. Year-to-year differences in chemistry within one watershed are also highly variable and difficult to generalize without sufficient record (Schindler et al. 1976).

Studies reported here were conducted on two experimental watersheds in the Experimental Lakes Area of northwestern Ontario (Lat. 49°40′ Long. 93°44′) which were monitored for 4 yr before they were severely damaged by a windstorm and burned almost exactly 1 yr later in an uncontrolled forest fire. An adjacent

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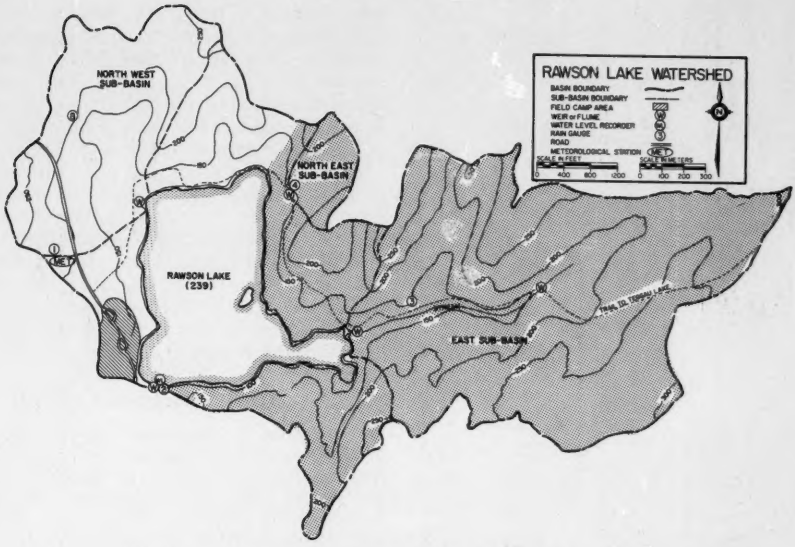


FIG. 1. Rawson Lake (Lake 239) and its drainage, showing the locations of the three subbasins studied. Areas burned by the forest fire are shaded. Locations of hydrological and meteorological equipment are also shown. Contour intervals are in feet, relative to the lake surface, which is 100.0 ft (30.5 m) (assumed).

third experimental watershed, monitored for the same period, was only slightly wind damaged and was unburned (Fig. 1). The unique opportunity afforded by these "before and after" studies allows us to make detailed interpretations without having to consider the differences in soils, geology, or vegetation, which may be sources of considerable error when "side by side" basins are compared.

The area burned is in the Great Lakes–St. Lawrence Forest Region (Rowe 1972). The forest community in all three watersheds was essentially similar, characterized by commercially valuable mature stands of jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* Mill. of 80–100 yr old) with isolated groves of deciduous species, white birch (*Betula papyrifera* Marsh.) and trembling aspen (*Populus tremuloides* Michx.).

Windstorm and Fire Conditions

At 05:00 on July 7, 1973, a severe windstorm struck the experimental watersheds. For a period of 20 min, gusts in excess of 150 kph uprooted trees or snapped them off above the ground. Up to 50% of the trees in valley bottoms and 100% of the trees on hilltops were blown down in a 350 km² area. In our three experimental watersheds, estimated blowdown ranged from less than 20% in the Northwest Subbasin to 70–100% in the East Subbasin (Ontario Ministry of Natural Resources, unpublished maps).

Almost exactly 1 yr later, at 12:08 on June 26, 1974, following 2 wk of low humidity, strong winds, and warm temperatures, a man-caused industrial fire was set accidentally in an area 3 miles (4.8 km) southeast of our experimental watersheds. Driven by a southeast wind in "extreme" fire weather, the fire spread quickly in the dry year-old windfall, reaching the experimental watersheds by late afternoon. Flames 15–20 m in height and a smoke column rising to over 8000 m were observed. The East and Northeast Subbasins were burned completely, while the Northwest Subbasin was left untouched as the westerly course of the fire was checked at a hastily cleared fireline separating the Northeast and Northwest Subbasins.

The fire (known as Dryden No. 16) was extremely hot. The flame-front intensity (I) was estimated to be 13,000 kW/m as calculated after Byram (1959), with conversion to metric units (Van Wagner 1978):

$$(1)\quad I = H \times w \times r$$

where H is the fuel low heat of combustion (KJ/kg), w is the fuel consumed (kg/m²); and r is the forward rate of spread (m/s).

Virtually all vegetation less than 2.5 cm in diameter was consumed. In the most intensely burned areas, organic matter in surface soils was mineralized to a depth of several centimetres, and the surface of exposed or thinly-covered bedrock was shattered. The fire-resistant cones of jack pine were consumed in many

TABLE 1. Annual precipitation (ppt) and water yields in metres from three stream basins. 1970–72 figures are means and 95% confidence limits. Values outside the 95% confidence limits are underlined.

	Northwest (Control)				Northeast (Burned)				East (Burned)			
	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2
(1) ppt	0.824±0.268	0.858	0.679	0.636	0.824±0.268	0.858	0.679	0.636	0.824±0.268	0.858	0.679	0.636
(2) runoff	0.314±0.233	0.323	0.218	0.173	0.364±0.269	0.692	0.422	0.353	0.281±0.072	0.448	0.374	0.286
(3) runoff/ppt	0.381±0.235	0.376	.321	0.272	0.442± .036	0.803	0.618	0.555	0.341±0.048	0.522	0.551	0.450
(4) 3 as % of control mean	100±62	99	84	71	100±8	182	140	126	100±14	153	162	132

areas because of their close proximity to the ground. The fire occurred after most herbaceous plants had flowered. Little precipitation fell in July and early August following the fire. As a result, there was very little revegetation of the basins. Two subsequent rainstorms of slightly more than 5 cm each, occurred in mid and late August. The area burned, near the Experimental Lakes Area camp (Fig. 1), has only thinly distributed overburden, and is typical of the more fragile parts of the boreal forest. Conditions were therefore optimal for high nutrient losses.

The final size of Fire 16 was 1457 ha. A much larger fire, Dryden No. 18, was started by lightning on June 29, burning 323 km^2 directly east of and adjoining the area. This fire, in similar terrain and vegetation, has been analyzed by Stocks (1975).

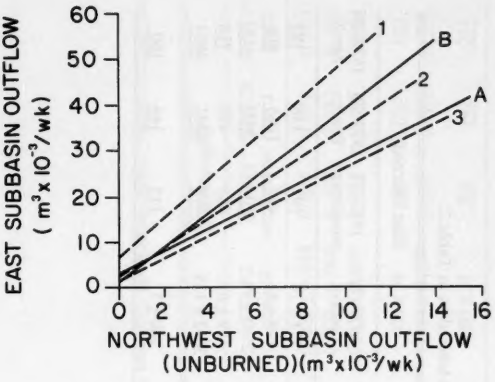
Data Collection and Analysis

Flows from the Northwest and East Subbasins were measured continuously with weirs and stage recorders. The Northeast Subbasin was metered manually to obtain a continuous flow record. Chemical analyses were done at least weekly, and up to several times per day during the first large storms after the fire. Hydrological and chemical methods were discussed by Schindler et al. (1976). Only results for nitrogen, phosphorus, and potassium, elements of special interest to biologists, are reported here. We reasoned that total watershed yields could be due either to increased water flow, or increased chemical concentration, or both. Dividing total annual yield by annual water flow gives a mean chemical concentration, which allows the effects of flow to be distinguished from increased concentration. Total annual yields and mean annual concentrations were calculated for each of the "pre-impact" calendar years, 1970, 1971 and 1972, as well as the three "post-impact years." July 1973 through June 1974 is referred to as "windstorm year," July 1974 through June 1975 as "fire year 1" and July 1975 through June 1976 as "fire year 2." Mean annual yield and 95% confidence limits were calculated for the pre-fire years. Annual precipitation was treated in the same manner.

Precipitation and Runoff

Precipitation in the post-impact years was within the 95% confidence envelope generated from pre-impact data (Table 1). Runoff and runoff/precipitation ratios were also within these limits for the unaffected Northwest Subbasin, with the ratios decreasing in the drier years of 1974, 1975, and 1976. In the blowdown and burned basins the ratios increased initially to 1.6× and 1.8× the pre-impact values in spite of the drier years and then followed a rapidly decreasing trend similar to that of the unburned basin.

The surprisingly short period of recovery of the pre-fire hydrological characteristics of the burned basins is illustrated in Fig. 2, showing the relative yield of the East and Northwest Subbasins under coincident rainfall conditions. Prior to the impacts, the larger East Subbasin produced approximately 2.5× the stream outflow of the Northwest Subbasin in larger and more



CURVE	PERIOD	NO. OF WEEKS	INTER-CEPT	SLOPE	COEFF OF CORR.
A	PRE-IMPACT	34	2980	2·51	·94
B	WINDSTORM YEAR	20	1048	3·79	·94
1	FIRE YEAR 1	20	6333	4·35	·91
2	FIRE YEAR 2	29	2207	3·26	·88
3	FIRE YEAR 3*	17	1407	2·46	·68

*partial year to October 1976

FIG. 2. Relationship of measured weekly streamflow volumes between the unburned Northwest Subbasin and the East Subbasin from 1971 to 1976 during the open water season under coincident rainfall conditions. Segments of the period are represented by Curve A: pre-windstorm, Curve B: year following the windstorm, Curve 1: first year following the fire in the East Subbasin, Curve 2: second year following the fire, Curve 3: third partial year following the fire ending in October 1976. The pre-impact relationship between basin outflows (volumetric) was re-established in three growing seasons.

prolonged storm runoff hydrographs and higher "base" flows between storm runoff events. The relative yields are consistent with the relative basin areas of 170.3 ha (East) and 62.8 ha (Northwest) and the large groundwater storage potential of the East Subbasin. In the year following the windstorm, the relative yield from the East Subbasin increased by 50% during periods of storm runoff. In the first year following the fire, the relative yield increased by 70% primarily in the form of a higher base flow between storm events. However, within 2 yr, in fire year 3, the relative yield had decreased to its pre-impact value. From these data it is apparent that the period of excess runoff following a fire is relatively short (3 yr), corresponding to the rapidly developed evapo-transpiration demand of the flourishing post-fire vegetative cover. A study of changes in the relative base flows and storm hydrograph peaks and durations is presently being undertaken by R. W. Newbury and K. G. Beaty (Freshwater Institute, Winnipeg).

Nitrogen Yields

Yields from the unaffected Northwest Subbasin in the "post-impact years" were within the 95% confidence limits generated from "pre-impact" years (Table 2). The one exception, suspended particulate N, is near the lower confidence threshold for 1975–76, the driest year in recent history.

In contrast, yields of all nitrogen fractions from both of the affected watersheds were significantly higher in the windstorm year. After the burn, all fractions were higher than background in the East Subbasin and all but suspended particulate nitrogen were higher in the Northeast Subbasin. Greatest increases were for nitrate in the year after the burn, with annual yields 3.4 and 9× higher than pre-impact means. Yields of total nitrogen were roughly 2× higher than normal in both basins in all of the disaster years.

Mean annual concentrations did not increase as much as total yields, although values for all dissolved fractions and total nitrogen were significantly higher in the post-impact years in both of the affected basins (Table 3). Total dissolved nitrogen concentrations were also significantly higher in post-fire years in the unaffected basin. This may be due to the lower water yields in the latter years.

Suspended particulate nitrogen concentrations in some post-impact years were not significantly higher, indicating that most of the effect observed in yield was due to the increased water flow.

Phosphorus Yields

The pattern of total yields for phosphorus is very similar to that observed for nitrogen. Losses of both suspended and dissolved fractions as well as total phosphorus increased 1.4 to 3.2× in the post-impact years (Table 4).

Some differences are noted in mean annual concentration. The windstorm did not cause significant increases in concentration of any phosphorus fraction in the East Subbasin, while in the Northeast Subbasin a 2.5× increase in suspended P was recorded (Table 5). The fire caused increases of 2–2.4× in the Northeast Subbasin, and 1.1–1.5× in the East Subbasin. Concentrations in the East Subbasin did not increase significantly until the second year after the fire.

Potassium Yields

With a few exceptions, both annual yields and mean annual concentrations of potassium were higher after the impacts (Tables 4 and 5). As for phosphorus and nitrogen, increases were in the 1.4–2.9× range.

Discussion

In studies of the Little Sioux Fire in the nearby Boundary Waters Canoe Area, McColl and Grigal (1977) and Wright (1976) concluded that the con-

TABLE 2. Annual yields (kg/ha) of nitrogen from three stream basins. 1970–72 figures are means and 95% confidence limits. Values outside the 95% confidence limits are underlined.

Element	Northwest (Control)				Northeast (Burned)				East (Burned)			
	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1971–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2
(1) NH_4-N	0.033±0.022	0.051	0.039	0.019	0.057±0.009	0.183	0.128	0.158	0.038±0.009	0.103	0.087	0.082
(2) NO_3-N	0.052±0.034	0.080	0.054	0.025	0.027±0.001	0.060	0.093	0.044	0.068±0.016	0.221	0.609	0.403
(3) Tot. Diss. N	0.888±0.281	0.926	0.835	0.684	1.283±0.032	2.559	2.191	2.746	0.874±0.032	1.573	2.177	1.860
(4) Susp. partic. N	0.141±0.062	0.125	0.104	0.078	0.339±0.083	0.882	0.384	0.372	0.160±0.021	0.253	0.270	0.323
(5) Σ N	1.029±0.343	1.051	0.939	0.762	1.622±0.115	3.441	2.575	3.118	1.034±0.053	1.826	2.447	2.183
(6) 5 as % of control mean	100±33	102	91	74	100±7	212	159	192	100±5	177	237	211

TABLE 3. Mean annual concentrations (mg/m³) of nitrogen from three stream basins. Columns are labeled as in Table 2.

Element	Northwest (Control)				Northeast (Burned)				East (Burned)			
	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1971–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2
(1) NH_4-N	105±70	158	179	110	157±25	264	303	448	111±26	197	158	182
(2) NO_3-N	166±108	248	248	145	74±3	87	220	125	199±47	423	1105	896
(3) Tot. Diss. N	2828±895	2867	3830	3954	3525±88	3698	5192	7779	2563±94	3013	3951	4133
(4) Susp. Partic. N	449±197	387	477	451	931±228	1275	910	1054	469±62	485	490	718
(5) Σ N	3277±1092	3254	4307	4405	4456±316	4973	6102	8833	3032±155	3498	4441	4851
(6) 5 as % of control mean	100±33	99	131	134	100±7	112	137	198	100±5	115	146	160

TABLE 4. Annual yields (kg/ha) of phosphorus and potassium from three stream basins. 1970–72 figures are means and 95% confidence limits. Values outside the 95% confidence limits are underlined.

Element	Northwest (Control)				Northeast (Burned)				East (Burned)			
	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1971–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2
(1) Tot. Diss. P	0.032±0.020	0.022	0.020	0.013	0.061±0.022	0.100	0.169	0.180	0.036±0.014	0.054	0.053	0.054
(2) Susp. Partic. P	0.019±0.012	0.010	0.008	0.006	0.032±0.001	0.103	0.043	0.036	0.020±0.005	0.028	0.032	0.030
(3) Σ P	0.051±0.032	0.032	0.028	0.019	0.093±0.023	0.203	0.212	0.216	0.056±0.019	0.082	0.085	0.084
(4) 3 as % of control mean	100±63	63	51	37	100±25	218	228	232	100±34	146	152	150
(5) Diss. K	0.778±0.334	1.014	0.802	0.603	1.621±0.068	3.010	4.263	3.021	1.514±0.323	3.151	4.425	3.239
(6) 5 as % of control mean	100±43	130	103	78	100±4	186	263	186	100±21	208	292	214

TABLE 5. Mean annual concentrations (mg/m³) of phosphorus and potassium from three stream basins. Columns are labeled as in Table 4.

Element	Northwest (Control)				Northeast (Burned)				East (Burned)			
	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1971–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2	1970–72 control	1973–74 windstorm	1974–75 fire 1	1975–76 fire 2
(1) Tot. Diss. P	102±64	68	92	75	168±60	145	400	510	128±50	121	142	189
(2) Susp. Partic. P	61±38	31	37	35	88±3	149	102	102	71±18	62	86	105
(3) Σ P	162±102	99	128	110	255±63	293	502	612	199±68	183	227	294
(4) 3 as % of control mean	100±63	61	79	68	100±25	115	197	240	100±34	92	114	148
(5) Diss. K	2478±1064	3139	3679	3486	4453±187	4350	10102	8558	5388±1149	7033	11832	11325
(6) 5 as % of control mean	100±43	127	148	141	100±4	98	227	192	100±21	131	220	210

centrations of nutrients did not increase in streams following the fire, even though soil and groundwater concentrations of nutrients increased. There are a number of possible reasons for this difference from our studies.

The above authors had no pre-fire data for their basins. Our previous work (Schindler et al. 1976) and the pre-fire data presented in Tables 1 to 5 indicate that there are differences of at least 2× in both year-to-year and basin-to-basin variation in nutrient yields. It is therefore difficult to distinguish the effects of fire from variation under nonfire conditions. The later occurrence and much more intensive burning of the Experimental Lakes Area fire would also have contributed to the difference. The Little Sioux Fire occurred early in the spring, before the flowering of major herbs in the area. It did not appear to have burned as intensely or as thoroughly as the Experimental Lakes Area fire described above. In striking contrast to the slow recovery of terrestrial vegetation in the first year following the Experimental Lakes Area fire, the Little Sioux watersheds had extensive herbaceous ground cover within a few weeks.

For most nutrients, wind damage to the forest caused effects as severe as a forest fire, even though understory vegetation was intact in the former case and totally destroyed in the latter.

After the fire, annual losses of phosphorus and nitrogen from watersheds via the streams were less than 40% of the annual inputs from rain, snow, and dustfall (Schindler et al. 1976). Even with short-term increases in water yields, there appears to be little likelihood that fire-caused losses of nutrients will significantly affect the regrowth of forests. On the contrary, the extensive remineralization of organic matter in soils during the fire left high concentrations of soluble reactive phosphorus and NH_4-N in the upper 5 cm, little of which was removed during subsequent rainfalls (D. W. Schindler and G. Morrison unpublished results).

There also appears to be little reason for fearing that the increased nutrient losses after a fire or windstorm will have adverse effects on receiving waters. The phytoplankton and nutrient concentrations in Rawson Lake have not increased detectably since the fire (D. W. Schindler unpublished data), although due to the long water renewal time of the lake (about 7 yr), maximum effect may follow maximum yield from the input stream by several years.

Our results indicate that in the absence of an unusually short fire interval, there are no chemical or limnological reasons for supporting fire suppression programs in similar areas of the boreal forest of North America.

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Water Renewal Efficiency of Watershed and Lake Combinations in the ELA Region of the Precambrian Shield

R. W. NEWBURY AND K. G. BEATY

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

NEWBURY, R. W., AND K. G. BEATY. 1980. Water renewal efficiency of watershed and lake combinations in the ELA region of the Precambrian Shield. Can. J. Fish. Aquat. Sci. 37: 335–341.

Individual components of lakewater budgets in the Experimental Lakes Area (ELA) are highly variable through time. Direct field measurements combined with empirical relationships for unmeasurable components are required to achieve balanced lakewater budgets over short intervals of several hours or days. However, annual variations in the hydrological regime are related to a few simple characteristics of the lake and surrounding watershed. In the 1969–77 period the annual ratios of terrestrial evapotranspiration/precipitation and lake evaporation/precipitation showed little variation and were of a similar magnitude. As a result, the potential for water renewal or replacement in the lake basins annually can be characterized by only four factors: the annual precipitation, the tributary drainage basin area, the lake area, and the lake volume. The potential has been expressed as the Annual Watershed Efficiency (AWE), the reciprocal of the water renewal time, to obtain a linear relationship between the four variables. The AWE i a measure of the extent to which a particular watershed and lake combination can renew the lake water under various annual precipitation conditions. A general graphical relationship for the AWE has been prepared for small headwater lakes similar to those of the ELA for a range of annual precipitations.

Key words: watershed hydrology, water renewal, water residence, precipitation/runoff ratios, water budgets, evaporation, watershed efficiency

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On observe de fortes variations temporelles des composants individuels des bilans d'eau des lacs dans la région des lacs expérimentaux (ELA). Pour en arriver à des bilans équilibrés d'eau de lac pendant de courts intervalles de plusieurs heures ou jours, il faut des mesures directes sur le terrain combinées avec des relations empiriques pour les composants non mesurables. Cependant, les variations annuelles du régime hydrologique sont liées à quelques caractéristiques simples du lac et du bassin qui l'entoure. Durant la période 1969–77, les rapports annuels évapotranspiration terrestre/précipitation et évaporation lacustre/précipitation accusent peu de variation et sont de même ordre de grandeur. Comme résultat, on peut caractériser par seulement quatre facteurs le potentiel de renouvellement ou de remplacement d'eau annuellement dans les bassins des lacs: précipitation annuelle, superficie du bassin hydrographique, superficie du lac et volume du lac. Le potentiel a été exprimé comme efficacité annuelle du bassin (AWE, «Annual Watershed Efficiency»), la réciproque du temps de renouvellement de l'eau, pour donner une relation linéaire entre ces quatre variables. L'AWE est une mesure du degré auquel une combinaison particulière de bassin hydrographique et de lac est capable de renouveler l'eau du lac dans diverses conditions de précipitation annuelle. On a préparé une relation graphique générale de l'AWE pour les petits lacs de tête des eaux semblables à ceux de la ELA et applicable à une variété de précipitation annuelle.

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THE long-term mass balance of the chemical constituents of lake water is governed by biological processes within a lake basin and by the transport of dissolved and particulate matter in the water entering and leaving the basin. Under the influence of man, the balance may be altered significantly. Chemicals are added in precipitation, runoff from urban, agricultural, or forestry areas, and by the direct addition of wastes. The balance is also altered by manipulating the natural water regime that sustains the lake. Storage and diversion projects for irrigation in the Missouri and Souris River basins in southern Canada and the generation of hydroelectric power in the Churchill and Nelson River basins in northern Canada are large-scale examples of headwater manipulations that alter the volume and timing of water flowing into downstream lakes. The determination of the diverse sources and destinations

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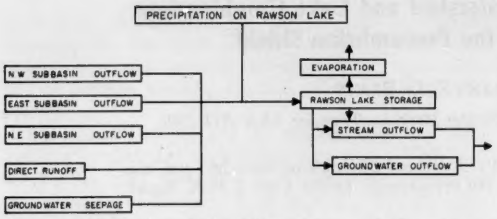

FIG. 1. Schematic relationship of water budget components for Lake 239 (Rawson Lake). The components are typical of the small ELA watershed and lake combinations.

of water in lake basins and their surrounding tributary areas is a primary step in assessing the effects of man-induced changes on the behavior and quality of lake waters. The whole-lake chemical and biological experiments undertaken at the Experimental Lakes Area (ELA) to simulate the ecological consequences of man-induced change are supported by parallel water budget studies of experimental and control lake basins. The continuous monitoring and balancing of the input and output components of lake water budgets facilitates the comparison of ELA lakes and provides a basis for transferring the ELA experience to other systems (Newbury et al. 1979).

Materials and Methods

In 1968, field studies were initiated to determine daily and weekly water budgets in a centrally located 350 ha index basin surrounding Rawson Lake (Lake 239) (Newbury and Beaty 1969–74). A rain gauge network, Class A weather station, stream gauging program, and a continuous level and outflow monitoring station were established with the assistance of the Atmospheric Environment Service of Canada (AESC) and the Water Survey of Canada (WSC). A network of groundwater piezometric stations was established in the 1969–73 period surrounding Rawson Lake under the direction of Dr J. Cherry of the University of Waterloo. Studies of the groundwater and streamflow relationships were subsequently undertaken in the Northwest and East subbasins tributary to Lake 239.

In addition to the Rawson Lake studies, a stream gauging program was initiated by the WSC below the outlets of several adjacent lakes (WSC 1972). By 1976, outflow recording stations were established below 14 lakes. The hydrometric and meteorological records of the ELA network are reported regularly in the AESC and WSC regional summaries (AESC 1969–77; WSC 1969–77). Water and chemical budgets for Rawson Lake in the 1969–73 period were summarized in Schindler et al. (1976).

Results and Discussion

Headwater Lake Hydrology

Water sources and destinations in a headwater lake system typical of the ELA are shown schematically in Fig. 1. Since measurements began in 1972, 53% of the total input to Rawson Lake has been contributed from three watershed areas (170.3, 62.8, and 9.6 ha) through gauged intermittent streams. Direct runoff across the shoreline boundary from the areas immediately adjacent to the lake (98.5 ha in total) contributed an estimated 17% of the inflows. Precipitation on the lake surface accounted for the remaining 30% of the water sources. The small amount of groundwater seepage to and from the lake was estimated as less than 1.5% of the surface flows due to the shallowness of the surficial deposits and the low relief of the bedrock-controlled basin (Kennedy 1974). Loss of water from the lake occurred as evaporation from the surface (21%) and as flow through the outlet stream (79%).

Over short intervals, changes in lake level and consequently water storage reflect the periodic and variable nature of the water sources. In a typical year over 50% of the streamflow into Rawson Lake occurs during snowmelt and rainstorm periods which occupy less than 13% of the time. As a result, all of the hydrologic components sustaining the lake must be evaluated directly to achieve a water balance for a specific time interval. Precipitation, streamflow, lake levels and storage changes, and snow accumulation in the basins can be measured directly but runoff from adjacent terrestrial areas and evaporation from the lake surface must be estimated from indirect measurements and empirical relationships. Standard techniques for predicting direct runoff and evaporation required modification before balanced water budgets were obtained for the ELA lakes.

Direct runoff — In the ELA, direct runoff occurs during and following snowmelt and rainstorm periods in the near surface layers of the surrounding terrestrial area. Where impermeable shorelines exist (bedrock, clay deposits, etc.), rivulets of water emerge on the surface of the backshore areas and flow directly into the lake. This process can be observed on valley side slopes in adjacent watersheds where the analogous near-surface flows are collected in a central stream channel before entering the lake. The rate of movement of water towards the stream or shoreline in the temporarily saturated surface layers is greater than the rate of deeper groundwater movement below the water table. The hydraulic conductivity of saturated surface moss layers in the valleys tributary to Rawson Lake was estimated to lie between 0.05 cm/s and 0.5 cm/s compared to a value of 0.0005 cm/s measured in the underlying Quaternary deposits (Bottomley 1974).

The near-surface and surface flows from the valleys are quickly conducted to the lake once they enter the stream channel. The maximum travel time measured by dye tracers from the upper reaches of the streams tributary to Rawson Lake is less than 6 h. Under these conditions, direct runoff from valley side walls can be assessed from the slightly delayed peak flow hydrograph recorded at the stream outlet following a storm. The baseflow, a longer-term streamflow component associated with deeper groundwater sources that occurs before and after the storm period, must be separated

from the recorded hydrograph to obtain the direct runoff component of the flow.

The direct runoff process in backshore areas adjacent to the lake is similar to the valley process without the period of travel in the stream or the base-flow component. Where stream records are available in nearby similar basins, the unmeasured direct runoff can be determined by extrapolating the direct runoff estimate from the separated storm hydrograph in proportion to the size of the drainage areas with allowance for minor variations in rainfall between areas.

Where no streams or streamflow records exist in a headwater area, a method of estimating runoff was developed from meteorological records based on the analysis of 38 storms and hydrographs in the Northwest stream tributary to Rawson Lake. In the Northwest Basin the volume of runoff associated with each storm ranged between 1 and 39% of the total rainfall volume. High storm yields (runoff/rainfall) occurred when the basin was saturated by an antecedent storm or by snowmelt water. Direct runoff that occurred in May following the April snowmelt period correlated strongly with storm rainfall in the relationship:

$$RO = 0.36\mathrm{RF} - 0.09 \quad \text{(May only)} \quad (r = 0.98,\ n = 7)$$

where RO = direct runoff (mm),

RF = total storm rainfall (mm),

r = coefficient of correlation.

In the total April–October open-water season, the correlation decreased ($r = 0.8$, $n = 38$) as moisture conditions in the watershed varied.

To improve the direct runoff estimate for midsummer storms, correlations were developed with rainfall and an antecedent moisture index (AMI) of the basin moisture conditions at the time of the storm (Newbury and Beaty 1977). The AMI is based on the difference between the precipitation and estimated evapotranspiration demand in the 4 wk prior to the storm. The net water available in each week is weighted in a series based on proximity to the time of the storm as follows:

$$\mathrm{AMI} = 0.8(RF_1 - E_1) + 0.8^2(RF_2 - E_2) + 0.8^3(RF_3 - E_3) + 0.8^4(RF_4 - E_4)$$

where:

AMI = antecedent moisture index (mm),

RFn = total rainfall in week n (mm),

En = total evapotranspiration in week n based on estimated open water evaporation adjusted by a seasonal coefficient of 0.8, 0.8, 0.8, 0.7, and 0.7 for the months June–October, respectively (mm).

The direct runoff expressed as a depth on the drainage area was then successfully correlated with the storm rainfall and the AMI at the time of the storm where:

$$RO = 0.21RF + 0.11\mathrm{AMI} - 4.06$$

The coefficient of multiple correlation is 0.93 for the 31 storms which occurred after May when moisture conditions were variable.

In the ELA basins the AMI technique for estimating runoff is applied only where water and chemical inputs are required for individual storms. For longer periods, water budgets are based on the local rainfall and extrapolation of the storm yields recorded in the Rawson Lake basin during the same rainfall period.

Evaporation — The residual term of the weekly water balance prepared for Rawson Lake provides an estimate of the remaining unmeasurable component of a lakewater budget, evaporation. The evaporation derived from the Rawson Lake balance was used to evaluate two commonly used methods of predicting evaporation that could be applied to adjacent lakes in the ELA: evaporation pan measurements and a mass transfer relationship based on diffusion at the lake surface.

Pan evaporation — For a unit area of water surface, the ratio between the monthly water balance evaporation for Rawson Lake and the sum of the monthly net water losses from a standard Class A evaporation pan at the ELA weather station was determined for the 1972 to 1976 period. The ratios or monthly pan coefficients first increased and then decreased as the open-water season progressed (Table 1). On a unit area basis more water evaporates from the pan than the lake in the early part of the season as the temperature of the smaller water mass in the pan exceeds the lake temperature. In the Fall cooling period, the relative evaporation rates are reversed as the larger heat storage capacity of the lake maintains a higher water temperature in the lake than in the pan. Unfortunately, the ratio of evaporations varied too widely on a monthly basis to predict accurately monthly evaporation by this simple method. Only the less variable pan coefficient for the open-water season is applied to ELA lakes.

Mass transfer relationship — Where water balances were required for periods of less than a whole open-water season, coefficients for the following traditional mass transfer relationship were derived using average recorded wind speeds, temperatures, and relative humidities and the water balance evaporation term for Rawson Lake:

$$E = 7(1 + 0.06V_8)(e_s - e_a) \quad (r = 0.80,\ n = 30)$$

where E = monthly evaporation (mm)

V_8 = average monthly wind velocity at 8 m (km/h)

e_s = mean monthly saturated vapor pressure at the water surface (mm Hg)

e_a = mean monthly ambient vapor pressure (mm Hg)

TABLE 1. Monthly evaporation and pan coefficients for Rawson Lake 1972–76.

Date	Water budget evaporation (mm)	Pan evaporation (mm)	Pan coefficient	SD
May	35.6	111.3	0.32	0.23
June	89.2	156.5	0.57	0.11
July	107.2	178.7	0.60	0.10
August	102.6	123.6	0.83	0.29
September	77.9	67.7	1.15	0.35
October	46.1	42.3	1.09	0.97
Open water season	458.6	716.6	0.64	0.17

TABLE 2. Monthly water/air temperatures differentials based on mean lake depth.

May	$\Delta T = 0.8 - 0.17\,\bar{d}$	($r = -0.62$)
June	$\Delta T = 3.6 - 0.11\,\bar{d}$	($r = -0.69$)
July	$\Delta T = 3.0 - 0.09\,\bar{d}$	($r = -0.43$)
August	$\Delta T = 2.8$	($r = 0.001$)
September	$\Delta T = 3.9 + 0.16\,\bar{d}$	($r = 0.57$)
October	$\Delta T = 2.6 + 0.12\,\bar{d}$	($r = 0.60$)

ΔT = mean monthly water surface temperature minus mean monthly ambient air temperature (°C)
$\bar{d}$ = mean lake depth (m)

The coefficient of 7.0 obtained using the Rawson Lake data falls below the value of 11 generally recommended for small lakes (Bruce and Clark 1966). The low value obtained for Rawson Lake may be attributed to the high proportion of the ELA covered by open water and the discrepancy in size between Rawson Lake and the larger lakes and reservoirs on which the general coefficients are based. The mass transfer relationship based on the Rawson Lake experience was subsequently adopted for estimating monthly evaporation on outlying lakes.

Lake surface temperature estimates — A further difficulty remains in the application of mass transfer and similar techniques to outlying basins. Regional wind velocities and vapor pressures are generally available but individual lake surface temperatures are not. The surface temperature on which the saturated vapor pressure in the mass transfer relationship is based varies widely between lakes of different morphometries. To overcome this difficulty, the water surface temperature recorded at nine ELA lakes of varying area and depth in the weekly sampling program (Newbury and Beaty 1977) were compiled and averaged for 260 open-water months in the 1969–76 period and correlated with observed average monthly air temperatures. For the relatively uniform ELA basins the differential between the mean monthly water surface temperatures and the ambient monthly air temperature varied with a simple measure of the lake geometry, the mean lake depth. As indicated in Table 2, the relationship shifts from a negative correlation early in May, June, and July to a positive correlation in September and October corresponding to the warming and cooling periods of the open-water season.

The use of predicted monthly water surface temperatures as compared to recorded values in the mass transfer relationship for Rawson Lake did not significantly reduce the correlation with the evaporation derived from the water balance ($r = 0.8$ to $r = 0.74$). The monthly differentials were subsequently adopted for predicting water surface temperatures for use in the mass tranfer relationship for monthly intervals on outlying lakes.

LONG-TERM HYDROLOGIC PROPERTIES OF ELA LAKES

The long-term hydrologic behavior of a lake and watershed combination is commonly characterized in limnological studies by the mean residence time of water in the lake basin (Vollenweider 1968; Schindler 1971; Dillon and Riger 1975). The residence time or water renewal time is derived from the ratio of the lake volume to the annual volume of outflow where outflow records or estimates are available.

Water renewal in ELA lakes — The outflow measured below 14 ELA lakes in the period from 1970 to 1977 and related lake and watershed characteristics are summarized in Table 3. For intervals of a year of more, short-term lake level changes and the storm by storm variability in runoff are not as significant in lake water balances. Lake outflow is primarily related to the total runoff from the watershed area tributary to the lake and the net gain or loss between the lake surface and the atmosphere through precipitation and evaporation. A measure of the relative potential for replacing the lake volume in individual lakes with similar watershed characteristics and meteorological inputs may be obtained by dividing the total tributary area (including the lake area) above the outflow gauging station by the lake volume. In effect, the tributary area per unit volume is a measure of the water replacement potential for each lake under similar precipitation and runoff conditions. For the ELA lakes, the tributary area per unit volume ranged from 0.3 m^2 per cubic metre in Lake 224 to 50.8 m^2 per cubic metre of Lake 470 (Table 3). Lake surface areas are included in the total tributary area because the excess of annual precipitation after evaporation has occurred on the lake surface is of similar magnitude to the fraction of precipitation occurring as runoff from terrestrial areas. In the Rawson Lake water balance, the portion of the annual precipitation occurring as runoff from terrestrial areas was 36%. The average annual excess of precipitation after evaporation on the lake surface for the same period was 31%.

In the ELA, the water renewal time increases rapidly

TABLE 3. Summary of hydrological data for ELA lakes in the period 1970–77.

Lake Number (1)	Area ha (2)	Volume $10^4 m^3$ (3)	Total tributary area to outflow[a] ha (4)	Area/ Volume (4) ÷ (3) m^{-1} (5)	Annual outflow[b] 1970 $10^4 m^3$ (6)	1971 $10^4 m^3$ (7)	1972 $10^4 m^3$ (8)	1973 $10^4 m^3$ (9)	1974 $10^4 m^3$ (10)	1975 $10^4 m^3$ (11)	1976 $10^4 m^3$ (12)	1977 $10^4 m^3$ (13)	Previously estimated water renewal time years[d] (14)	Average water renewal time years[e] (15)
114	12.1	20.7	57.0	2.8	—	13.9	12.1	12.8	—	10.9	4.7	—	4.1	1.5
120	9.3	70.4	69.7	1.0	—	—	—	15.4	—	—	—	—	11.0	4.2
223	27.3	195.1	260.0	1.3	—	—	—	—	—	—	22.2	28.1	—	3.3
224	25.9	300.5	97.5	0.3	—	—	—	—	—	—	—	—	—	12.5
225	4.0	6.3	30.5	4.8	—	—	—	—	—	—	—	2.1	—	0.9
226	16.1	96.1	97.2	1.0	—	—	18.6	22.7	24.1	19.7	10.6	—	—	4.2
227	5.0	22.1	34.4	1.5	15.5	10.2	8.4	8.4	7.6	7.7	4.0	6.9	7.8	2.8
230[c]	1.7	10.4	8.9	0.9	—	—	3.4	2.0	—	1.2	0.9	2.5	20.0	5.0
239[c]	54.3	591.0	395.5	0.7	147.5	134.6	98.3	104.5	132.9	93.4	58.4	86.6	20.0	6.0
240[c]	44.2	267.0	725.2	2.7	274.7	260.3	194.9	230.7	251.9	224.5	127.1	204.8	4.4	1.6
261	5.6	16.0	47.6	3.0	—	—	11.9	—	20.7	14.8	8.5	8.5	4.0	1.4
265	13.1	128.0	71.0	0.6	—	—	16.8	20.2	25.6	—	—	4.4	21.0	7.1
303	9.9	15.0	54.1	3.6	—	19.1	12.6	13.7	15.9	13.0	7.4	—	2.6	1.1
304	3.4	11.5	26.4	2.3	—	—	—	—	—	—	—	—	3.2	1.8
470	5.7	3.3	167.7	50.8	69.1	—	51.2	59.8	66.4	54.0	31.3	—	—	0.1

[a]Total tributary area includes the lake surface.

[b]Incomplete records or unavailable data are indicated thus —. In small basins where lake outflow ceased in mid-November due to channel icing, early spring flows were not recorded. Estimates of unrecorded flows do not exceed 10% of the total annual flow based on the behavior of adjacent larger basins with year round outflows.

[c]Part of the tributary watershed area was burned in a forest fire in 1974.

[d]The previous estimate was based on regional climatological summaries (Brunskill and Schindler 1971).

[e]The average water renewal time is derived from Fig. 3 using the mean annual precipitation of the ELA.

as the ratio of tributary area to lake volume falls below 2 ha per cubic metre as shown in Fig. 2. Only 3 yr of data are plotted to preserve the clarity of the graph. The years 1970 and 1976 were the wettest and driest years during the period of record with 96.7 cm and 49.6 cm of precipitation, respectively. All other water renewal values for the 1970–77 period are contained within the envelope of these two extremes. The long-term average annual precipitation for the area is 70.5 cm based on the 68-yr period of record at Kenora, Ontario, 50 km to the northwest of the ELA. Data for the 1972 period of 70.9 cm precipitation are plotted to represent the average year.

Although the data are widely scattered for the entire period of record, in an individual year the water renewal relationship approximates an equilateral hyperbola that is strongly correlated with the tributary area per unit volume of each lake. The hyperbolic shape of the curve defining these commonly used limnological parameters is primarily due to the appearance of lake volume in the numerator of the ordinate and in the denominator of the abscissa. The auto-correlation is reinforced by the linear relationship between the annual outflow expressed as a depth on the total tributary area and the annual precipitation where:

$$\frac{\text{annual outflow}}{\text{tributary area}} = 0.34\ (\text{annual precipitation}) + 0.053 \qquad (r = 0.43, n = 53)$$

This overall runoff relationship is based on 53 annual outflow records excluding those of Lakes 230, 239, and 240 after 1974 when an intense forest fire burned through part of their tributary watersheds (Schindler et al. 1980).

Watershed efficiency in flushing ELA lakes — The factors governing the water renewal characteristics of the ELA lakes from year-to-year can be individually distinguished in a simple linear form if the water renewal time in Fig. 2 is replaced by its reciprocal, the annual watershed efficiency (AWE) as shown in Fig. 3. The AWE is derived from the annual outflow divided by the lake volume expressed as a percentage. In effect, it is a measure of the efficiency of the watershed and meteorological conditions in replacing a specific lake volume. A 100% efficiency would replace the volume of a lake in 1 yr. Under average precipitation and evaporation conditions in the ELA, slightly more than 4 m^2 of tributary area are required to annually renew a cubic metre of lake volume.

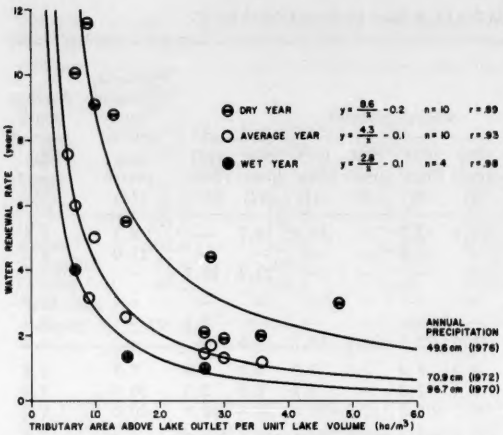

FIG. 2. The relationship of annual water renewal estimates to the tributary area per unit lake volume for 13 ELA lakes during the 1970–77 period. Lake 470 is not shown but is included in the correlation.

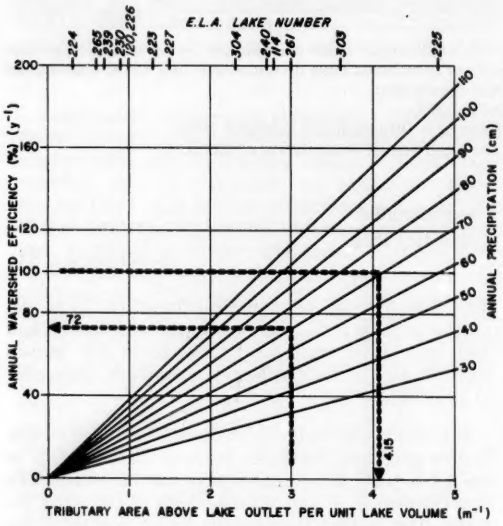

FIG. 3. General nomogram of Annual Watershed Efficiency in the renewal of lake water for the suite of ELA lakes during the 1970–77 period. A 100% efficiency indicates that the tributary area will provide sufficient water in a 1-yr period to potentially replace the lake volume for a given watershed and lake combination and a recorded or selected annual precipitation value. Under average precipitation conditions in the ELA watersheds, annual water renewal in a lake basin requires slightly more than 4 m^2 of tributary area for each cubic metre of lake volume.

To use the AWE nomogram for a headwater lake typical of the ELA, the tributary area above the lake outflow and the lake volume are first determined from topographic maps and bathymetric surveys. Precipitation is then recorded or a characteristic precipitation value is selected and the percentage of the lake water that will be renewed in 1 yr can be obtained directly from the graph. Using the average precipitation year and ELA Lake 261 with a tributary area/volume ratio of 3, the AWE derived from Fig. 3 is 72%; i.e. under average precipitation conditions, 72% of the lake water will be replaced in 1 yr by the particular watershed and lake area tributary to the outflow. Alternately, the average time of water residence in the lake is 1/0.72 or 1.4 years.

Average water renewal times based on the AWE nomogram are shorter than those previously predicted for several ELA lakes from regional climatic charts (Table 3, columns 14 and 15). The regional estimates were based on lower lake outflows produced by the assumption of higher evaporation rates and lower ratios of runoff/precipitation than were observed during the 1969–78 period.

Acknowledgments

The active support of many agencies and individuals was required to maintain 10 years of hydrologic record at the ELA. The authors wish to acknowledge the assistance provided by the Water Survey of Canada, the Atmospheric Environment Service, and the Groundwater Subdivision of Environment Canada. A major contribution to the studies was made by Dr John Cherry of the University of Waterloo and his graduate students in providing valuable groundwater analyses. The continuity of winter records is due primarily to the cooperation and persistence of Mr W. Forbes of the regional WSC office and the ELA camp managers: Messrs H. Schneider, P. Micaellis, and J. Beaty. Dr D. Schindler provided the right combination of advice and encouragement that have made the studies significant to the ELA program and a pleasure to perform.

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Experimental Acidification of Lake 223, Experimental Lakes Area: Background Data and the First Three Years of Acidification

D. W. SCHINDLER AND R. WAGEMANN

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

R. B. COOK

Lamont–Doherty Geological Observatory of Columbia University, Palisades, NY 10964, USA

T. RUSZCZYNSKI AND J. PROKOPOWICH

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

SCHINDLER, D. W., R. WAGEMANN, R. B. COOK, T. RUSZCZYNSKI, AND J. PROKOPOWICH. 1980. Experimental acidification of Lake 223, Experimental Lakes Area: background data and the first three years of acidification. Can. J. Fish. Aquat. Sci. 37: 342–354.

Sulfuric acid was used to add 1.60 eq·m^{-2} of hydrogen ion ([H^+]) to Lake 223 over a 3-yr period from 1976 to 1978, to simulate the effects of acid precipitation on chemical and biological characteristics of the lake. The pH of epilimnion water was lowered from 6.7 to 7.0 in 1976, to 6.0–6.2 in 1977, and to 5.7–5.9 in 1978. The effectiveness of acid addition at depleting alkalinity from the water column in the first 2 yr was 31–38%. As a result, the pH of the lake did not decrease as much as predicted from theoretical calculations. The low efficiency of acidification appeared to be due to generation of dissolved inorganic carbon (DIC) by sulfate reduction in anoxic regions of the hypolimnion. Sulfate reduction increased as addition of sulfuric acid caused the concentration of sulfate in the lake to increase. Mobilization of iron from hypolimnion sediments during anoxic periods caused DIC respired by decomposers to be accumulated as HCO_3^- to balance the Fe^{+2} charge, buffering against acidification of the lake. Most of the reduced sulfur was precipitated from the lake as FeS. As a result, H_2S in the hypolimnion was usually undetectable. Concentrations of ammonia, iron, manganese, zinc, and aluminum were higher in 1977 than in 1976. The transparency of the lake increased, although there was no apparent change in chlorophyll, dissolved color, dissolved organic carbon, or primary production.

Key words: lake acidification, acid rain, mass balance, water chemistry, sulfur cycling

SCHINDLER, D. W., R. WAGEMANN, R. B. COOK, T. RUSZCZYNSKI, AND J. PROKOPOWICH. 1980. Experimental acidification of Lake 223, Experimental Lakes Area: background data and the first three years of acidification. Can. J. Fish. Aquat. Sci. 37: 342–354.

Avec de l'acide sulfurique, on a ajouté 1,60 éq·m^{-2} d'ion hydrogène ([H^+]) au lac 223 pendant une période de 3 ans, de 1976 à 1978, dans le but de simuler les effets de la précipitation acide sur les caractéristiques chimiques et biologiques du lac. Le pH de l'eau de l'épilimnion passa de 6,7–7,0 en 1976 à 6,0–6,2 en 1977, et à 5,7–5,9 en 1978. L'abaissement de l'alcalinité dans la colonne d'eau pendant les deux premières années par addition d'acide s'est produit avec efficacité de 31 à 38%. Comme résultat, le pH du lac ne diminua pas autant que prédit d'après les calculs théoriques. Cette faible efficacité de l'acidification semble être due à la production de carbone inorganique dissous (DIC) par réduction des sulfates dans les régions anoxiques de l'hypolimnion. La réduction des sulfates augmente à mesure que l'addition d'acide sulfurique entraîne une augmentation de concentrations de sulfates dans le lac. La mobilisation du fer depuis les sédiments de l'hypolimnion en périodes anoxiques cause une accumulation, comme HCO_3^-, de DIC respiré par les décomposeurs pour équilibrer la charge de Fe^{+2}, agissant comme tampon contre l'acidification du lac. La plus grande partie du soufre réduit précipite du lac sous forme de FeS. Il en résulte que H_2S dans l'hypolimnion est ordinairement indétectable. Les concentrations d'ammoniac, fer, manganèse, zinc et aluminium étaient plus fortes en 1977 qu'en 1976. La transparence du lac augmenta, bien qu'il n'y ait pas eu de changement apparent de chlorophylle, de couleur dissoute, de carbone organique dissous ou de production primaire.

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ACID precipitation has severely affected freshwaters in many noncalcareous regions of the world, including eastern Canada (Beamish and Harvey 1972), the U.S. (Likens and Bormann 1974), and Scandinavia (Wright and Gjessing 1976). While the consequences of acid precipitation are known to include elevated concentrations of heavy metals and decreased diversity of aquatic biota (Gorham 1978), the mechanisms involved are poorly understood.

Quantitative data on input of acid and its fate within the aquatic system are not available for polluted sites, because of the great spatial and temporal variability in atmospheric deposition, the difficulty of estimating it, and the paucity of data on how it is modified during passage through terrestrial watersheds. As a result, it is still impossible to predict the rate at which lakes will become acidified when the hydrogen ion concentration in precipitation is increased. It is not known to what extent sediments or chemicals other than species of dissolved inorganic carbon buffer lake systems.

The increase in heavy metals that has been observed to accompany acidification (Dickson 1975; Wright and Gjessing 1976; Schofield 1976; Dillon et al. 1978) has been attributed to atmospheric fallout, or to metals leached by acid precipitation from soils in the watersheds of the lakes. The possibility that metals are mobilized directly from lake sediments as pH decreases does not appear to have been considered, yet many heavy metals which are mobilized by acid are concentrated in lake sediments, suggesting that sediments could be an important source. Schindler et al. (1980) have shown that aluminum, zinc, manganese, and iron are released from lake sediments in large-scale tube experiments, but the effect on a whole lake is unknown.

This study attempts to quantify the rate of acidification of a lake with known hydrology in response to a known input of acid, and to obtain detailed quantitative information about the effect of acidification on the dissolved inorganic carbon and heavy metal cycles. In related studies, the populations of algae and animals in the lake and their physiological responses to acidification have been measured (Malley 1980; Müller 1980; Schindler et al. 1979; E. J. Fee unpublished data).

Description of the Lake

Morphometric data for Lake 223 and a morphometric map are shown in Fig. 1. The lake, located in the Experimental Lakes Area (ELA) of northwestern Ontario, is typical for lakes of this size in the Precambrian Shield. A single inlet and outlet, each monitored by a V-notch weir serviced by Water Survey of Canada, supply and drain the lake. There are no areas of significant input or loss of groundwater. The watershed of the lake is of Precambrian gneisses and granites, forested by typical boreal pine–spruce forest. Brunskill and Schindler (1971) give a more detailed description of typical watersheds in the area.

The chemistry of the lake is also about average for lakes of the area (Prokopowich 1979, appendix 1). Water renewal time (τ = Volume of lake ÷ volume of annual outflow) for the lake were 9.4 and 6.9 yr in 1976 and 1977, respectively.

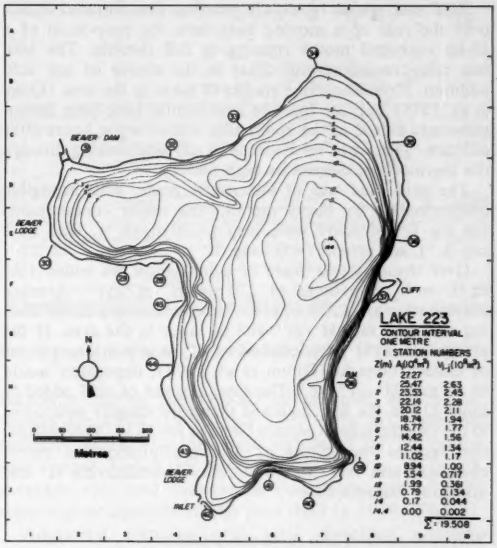

FIG. 1. Morphometric map of Lake 223. The outlet is near the beaver dam in the northwest corner.

Materials and Methods

BACKGROUND DATA

All sampling was done at one station located at the deepest point in the lake. In 1974 and 1975, the lake was sampled at 4-wk intervals during the ice-free season. Analyses were performed at eight depths in 1974. In 1975, samples were integrated, weighting the volume of sample at each depth for the volume of each layer sampled. Duplicate analyses were done for whole-lake average concentration, and for epilimnion average concentration.

No trace metal or color measurements were made in 1974 and 1975.

ACIDIFICATION REGIME

The acidification regime was designed to reduce the pH of the lake to a predetermined value early in each ice-free season, holding it at each value until the following spring, so that the physiological responses of various organisms and changes in sediment–water equilibria could be measured under conditions of known acidity. This regime also simulates roughly the natural pattern of a large pulse of acid during spring snowmelt, followed by periodic additions by summer rains. (Reviewed by Jeffries et al. 1979). In 1976, from 6 to 12 carboys (34 kg each) of acid were added each week to reduce the buffering capacity of the lake. In the spring of 1977, enough acid was added to lower the pH to approximately 6.0. In 1978, the pH was lowered to 5.75. Weekly additions of acid were made throughout the summer to hold the pH near the desired value.

Acid was added by slowly pouring concentrated H_2SO_4 over the rear of a moving boat, into the prop-wash of a 15-hp outboard motor running at full throttle. The lake was criss-crossed several times in the course of any acid addition. Physical mixing studies of lakes in the area (Quay et al. 1978) indicate that the acid should have been homogeneously mixed in the epilimnion within a few hours after addition. There was no evidence of any acid sinking through the thermocline, despite its high density.

The acid used was of electrolyte grade, with negligible contamination by heavy metals. The major contaminants (in $mg \cdot L^{-1}$ of acid) were iron ($<20\ mg \cdot L^{-1}$), lead ($<1\ mg \cdot L^{-1}$), and arsenic ($<0.2\ mg \cdot L^{-1}$).

Over the first two years of acidification we added 1.14 eq $H^+ \cdot m^{-2}$, an average of 570 meq $H^+ \cdot m^{-2} \cdot yr^{-1}$. Average precipitation at ELA is of pH 4.95 representing a direct loading rate of 8 meq $H^+ \cdot m^{-2} \cdot yr^{-1}$ to lakes in the area. If the precipitation pH were lowered to 4.1, as it is in many areas of eastern Ontario (Dillon et al. 1978), deposition would be 64 meq $H^+ \cdot m^{-2} \cdot yr^{-1}$. The total amount of acid added to Lake 223 in the first 2 yr was therefore roughly equivalent to moving the lake to eastern Ontario for 18 yr. This calculation ignores the effects of acid precipitation on runoff chemistry and the effect of outflow on removing H^+ and alkalinity from the lake.

Water Sampling and Analysis During Years of Acidification

In 1976, 1977, and 1978, samples were taken at depths of 1, 4, 8, 12, and 14 m once each month, using a peristaltic pump and polyethylene tubing. The epilimnion was sampled every second week in the ice-free season. pH samples were taken weekly or more often. All chemical analyses were performed as described by Stainton et al. (1977), except for trace metals. pH measurements were made at 20–22°C with a Radiometer model 29 pH meter and combination electrode, standardized with Radiometer or Fisher buffers of pH 5, 6, and 7. Bicarbonate concentrations in all years were calculated from pH, temperature, and dissolved inorganic carbon (DIC) data, using the dissociation constants of Garrels and Christ (1961). DIC analyses were performed conductimetrically. The coefficient of variation of replicate analyses was ±3%.

Trace metals were analyzed from samples taken in specially cleaned polyethylene bottles, filtered the same day in a teflon-coated apparatus on precleaned millipore membranes of 0.22-μm pore diameter, then preserved with 1 mL of redistilled HNO_3/L of sample. Analysis was by flameless atomic absorption, using a Perkin-Elmer HGA2100 furnace and 403 spectrophotometer.

Inflows and outflows were measured continuously with V-notch weirs and continuous level recorders. The chemistry of these was measured once a week when streams were flowing.

Total calculations for 1978 are not reported, due to delays in data processing.

Results

Distribution with Depth and Seasonal Averages

The sulfuric acid added each spring and summer remained in the epilimnion of the lake until fall turnover (Fig. 2). There is, therefore, a lag of several months between addition of acid to surface waters and its effect on deep water in the lake. In 1976 and 1977 the weekly additions of acid caused a gradual increase in the sulfate concentration of the epilimnion. By the end of the summer of 1977, SO_4 concentrations in the epilimnion were 2.5× background values.

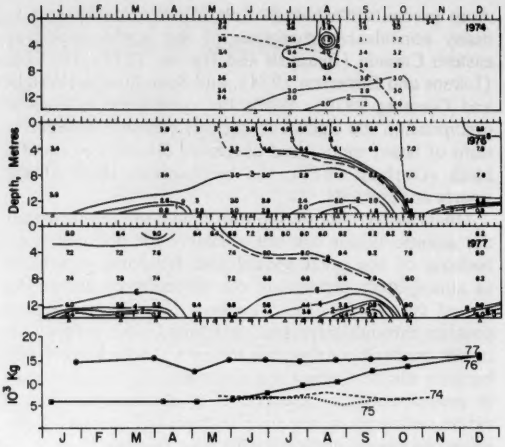

FIG. 2. The mass and distribution of sulfate in Lake 223, 1974–77. The upper three panels are isopleth maps for a preacidification season (1974) and the 2 yr of acidification (1976 and 1977). Data in the upper three panels are in mg $SO_4 \cdot L^{-1}$. The thermocline is shown as a dashed line. The lowest panel gives the total mass in the lake. Triangles are sampling dates. Arrows are acidification dates.

Sulfate concentrations in anoxic near-bottom waters in late winter and late summer were much lower than during the preceding period of overturn. Near-bottom concentrations of sulfate in late summer of 1977 were lower than in 1976, and in both years were lower than in 1974, even though spring sulfate concentrations in deep water were twice as high in 1977 as they were before acidification. Sulfate reduction appeared to be responsible. The reduced sulfur was seldom detectable as H_2S, either analytically or by odor. The highest concentration of H_2S–S observed was $400\ \mu g \cdot L^{-1}$ in September 1977. When detectable, values were usually below $100\ \mu g \cdot L^{-1}$.

The weekly additions of sulfuric acid severely depleted the bicarbonate content of the epilimnion (Fig. 3). Concentrations as low as 25–30 $\mu eq \cdot L^{-1}$ were reached by fall of 1976, and 5–10 $\mu eq \cdot L^{-1}$ was commonly observed during much of 1977. Bicarbonate concentrations in summer in the deepest part of the lake (12–14 m) were not depleted by acidification. The reasons for this will be discussed later.

Dissolved oxygen in the deep water of Lake 223 was depleted in all three summers and in both winters studied (Fig. 4). The apparent tendency for anoxic periods to become longer after acidification is discussed below.

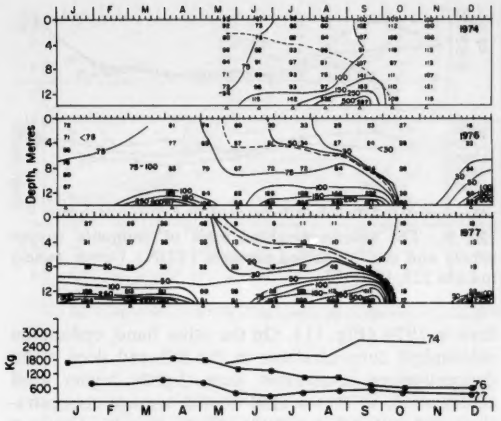

FIG. 3. The mass and distribution of bicarbonate in Lake 223, 1974–77. Panels are as in Fig. 2. Data in the upper three panels are in $\mu eq \cdot L^{-1}$.

Epilimnion pH values in 1974 were commonly 6.7 and greater. The highest value recorded was 7.0 in mid-September (Fig. 5). Acidification in 1976 scarcely lowered the average pH of the epilimnion. The highest value recorded was 7.20 and the average value for the summer was 6.79. In the summer of 1977, after buffering capacity had been reduced, epilimnion pH values were substantially lower, averaging 6.08, with a maximum of 6.60 and a minimum of 5.60. Despite the fact that acid added in 1976 was mixed throughout the lake at fall overturn, average deep water pH values in 1977 were only marginally lower than in 1976.

Iron concentrations in deep water increased substantially during the periods of summer and winter anoxia for 1974, 1976, and 1977 (Fig. 6). Seasonal patterns similar to this were exhibited by aluminum and copper during the period of acidification (Fig. 7). Average zinc and manganese concentrations in 1977 were higher throughout the year than in 1976 (Fig. 8).

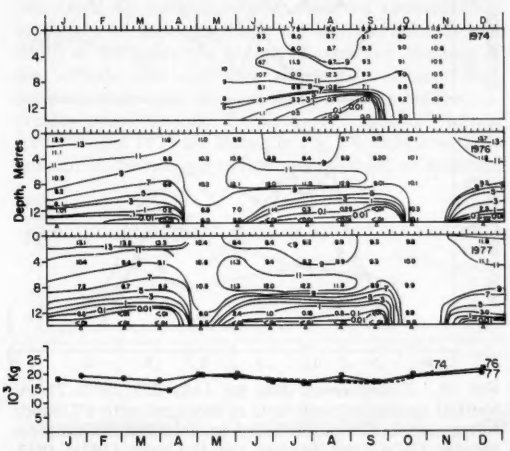

FIG. 4. Dissolved oxygen concentrations ($mg \cdot L^{-1}$) for Lake 223, 1974, 1976, and 1977. Panels are as in Fig. 2.

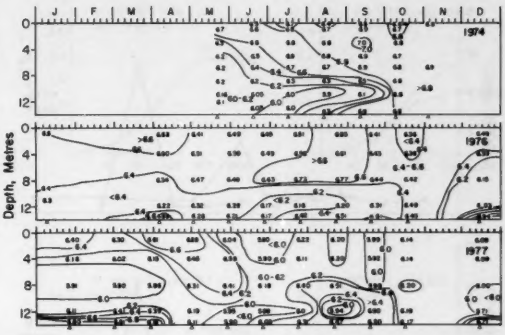

FIG. 5. pH isopleths for Lake 223, 1974, 1976, and 1977.

Ammonia nitrogen and total dissolved nitrogen (TDN) values were also high in anoxic regions of the lake. Average TDN concentrations in the lake during the summer of 1977 were slightly lower than in earlier years (Fig. 9). This may have been due to the more rapid water renewal of the lake or the deeper penetration of oxic waters at spring overturn during 1977, rather than to acidification.

No significant changes were detected after acidification in depth-distribution or whole-lake averages of the following elements: Ca, Mg, Na, K, Cl, suspended C, N, and P, dissoved organic carbon, total dissolved phos-

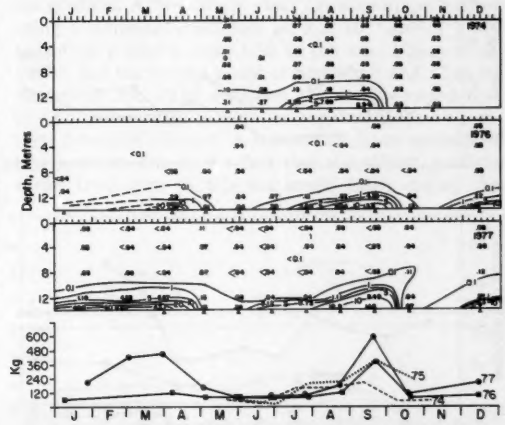

FIG. 6. The mass and distribution of total dissolved iron in Lake 223, 1974, 1976, and 1977. Panels are as in Fig. 2. Data in the upper three panels are in $mg \cdot L^{-1}$.

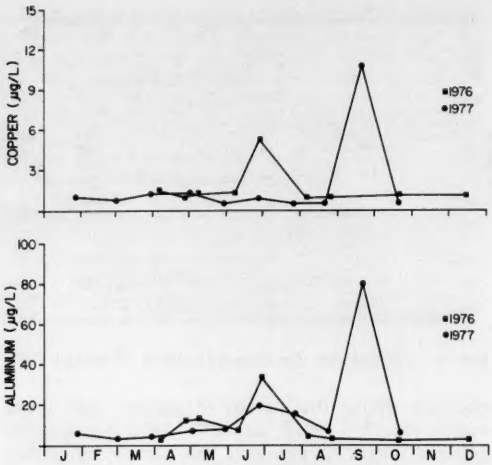

FIG. 7. The volume-weighted average concentration of dissolved copper and aluminum in Lake 223, 1976–77. A, Copper; B, Aluminum. (■ 1976; ● 1977.)

phorus, and reactive silicate. Cadmium, chromium, and lead were undetectable ($<1\ \mu g \cdot L^{-1}$).

The transparency of the lake, as measured by either Secchi depth or extinction coefficient, increased in 1976, and again in 1977 (Fig. 10; Shearer 1976; Shearer and DeClercq 1976, 1977, 1978). This was probably due to the fact that epilimnetic chlorophyll concentrations in midsummer of 1976 and 1977 were slightly lower

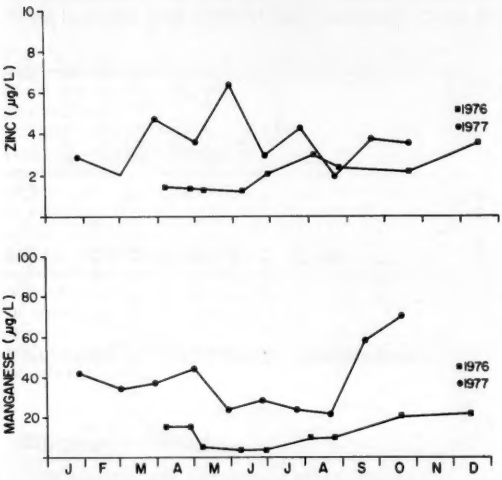

FIG. 8. The volume-weighted average concentration of zinc and manganese in Lake 223, 1976–77. A, Zinc; B, Manganese. (■ 1976; ● 1977.)

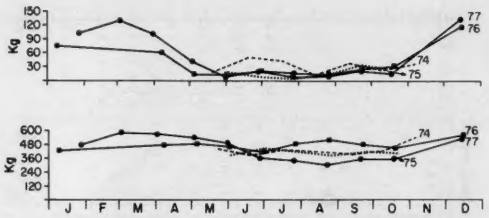

FIG. 9. The volume-weighted mass of ammonia (upper panel) and total dissolved nitrogen (TDN) (lower panel) in Lake 223, 1974–77.

than in 1974 (Fig. 11). On the other hand, epilimnion chlorophyll concentrations in the fall and deep water concentrations in summer were slightly higher after acidification, so that overall annual average concentrations did not differ greatly. There was no apparent change in the intensity of dissolved color, as measured by absorption at a wavelength of 425 nm (Fig. 12). No changes in phytoplankton species composition were apparent from routine counts, although a more thorough investigation of small, rare forms is under way (D. L. Findlay, Freshwater Institute, Winnipeg, personal communication).

Despite low concentrations of dissolved inorganic carbon (DIC), phytoplankton photosynthesis was not carbon limited. Carbon addition in short-term bottle bioassays appeared to cause a slight decrease in pro-

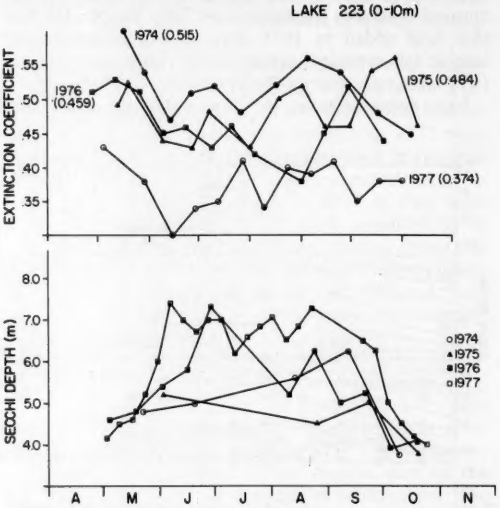

FIG. 10. Transparency data for Lake 223, 1974–77. A, Vertical extinction coefficients as measured with a Lambda Instruments Li-Cor model Li 185 quantum meter. Data from Shearer (1976) and Shearer and DeClercq (1976, 1977, 1978). Values in brackets are means for the ice-free season. B, Secchi disc transparency.

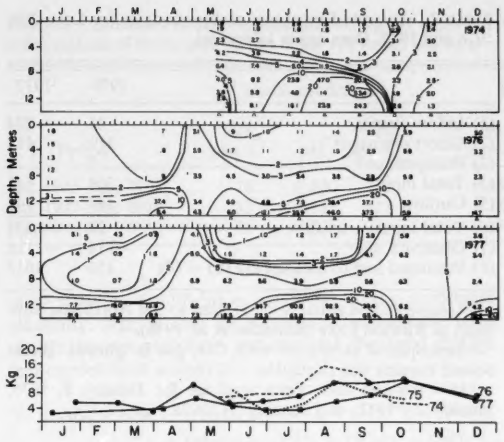

FIG. 11. The mass and distribution of chlorophyll in Lake 223, 1974, 1976, and 1977. Data in the upper three panels are in $\mu g \cdot L^{-1}$. Panels are as in Fig. 2.

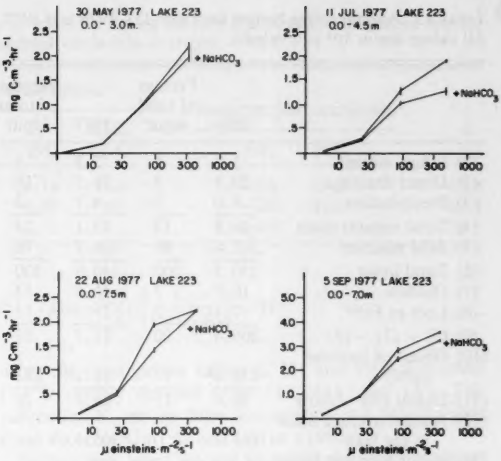

FIG. 13. Plots of photosynthesis vs. irradiance for bicarbonate enrichment (+ $NaHCO_3$) experiments with Lake 223 epilimnion samples. The DIC concentration ($mol \cdot L^{-1}$) was increased from 26 to 98, 40 to 156, 40 to 136, and 49 to 124 on May 30, July 11, August 22, and September 5, respectively. The vertical bars join the values obtained for the two replicates at each irradiance (Data from DeClercq and Shearer 1978).

duction, possibly as the result of a pH shift (Fig. 13). Annual phytoplankton production in 1976 and 1977 was within the variation expected for natural lakes in the area (Fee 1979 and E. J. Fee unpublished data).

MISSING SULFATE

Sulfate concentrations in the hypolimnion are greatly depleted during periods of anoxia in late winter and late summer (Fig. 2). This is undoubtedly due to the action of sulfate reducing bacteria. The amount of "missing" sulfate at a given depth on any day was calculated by subtracting the observed concentration of sulfate on that date from the concentration at that depth at the preceding period of overturn. Because of the sulfuric acid additions, the sulfate concentration increases each year. The amount of sulfate reduced increases proportionally. Isopleths of "missing" sulfate for 1976 and 1977 are shown in Fig. 14. Only a neglible amount of the missing sulfate was observed as dissolved H_2S. Therefore, all of it was assumed to have precipitated as FeS, as discussed later.

In addition, some sulfate would be expected to diffuse from the epilimnion into the hypolimnion. The sulfate gradient increases during both summer and winter due to hypolimnetic sulfate reduction. In summer, the addition of H_2SO_4 to the epilimnion will also increase the gradient. A "maximum case" calculation, performed using a diffusion coefficient of $5 \times 10^{-4}\ cm^2 \cdot s^{-1}$ obtained on a similar sized lake in the area (Quay et al. 1978) and the sulfate gradient between 9 and 12 m on September 19, 1977 indicates that a downward flux of 43 $mmol \cdot m^{-2} \cdot yr^{-1}$ might be expected if these conditions prevailed all year. A reasonable figure seems half that amount, due to the fact that the sulfate gradient varies from zero to this maximum figure during the

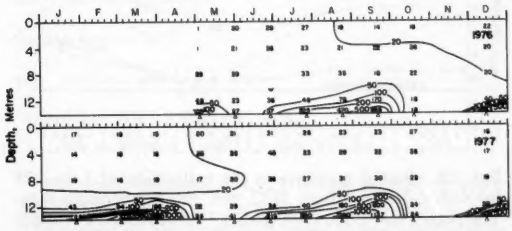

FIG. 12. Isopleths for absorbance by filtered lake water at 425 nm, Lake 223, 1976–77.

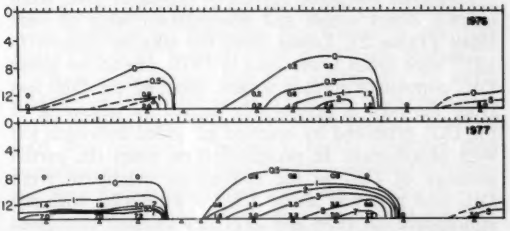

FIG. 14. Isopleths of "missing" sulfate, presumed sedimented as FeS. See text for methods of calculation. Values are in mg $SO_4 \cdot L^{-1}$.

TABLE 1. Annual sufate budget for Lake 223, 1976 and 1977. All values are in 10^3 equivalents.

	1976	Percent of total input	1977	Percent of total input
(1) Inflow stream	1.5	1	7.3	5
(2) Direct drainage	20.3	9	21.7	16
(3) Precipitation	5.0	2	4.1	3
(4) Total natural input	26.8	12	33.1	24
(5) Acid addition	203.4	88	106.9	76
(6) Total input	230.2	100	140.0	100
(7) Outflow	16.7	7	46.8	33
(8) Loss as FeS[c]	7.1	3	21.5	15
(9) (6) − (7) − (8)	206.4	90	71.7	51
(10) Observed increase (ΔM)[d]	178.0[a]	77	45.2[b]	32
(11) Deficit ((9) − (10))	28.4	13	26.5	19
(12) Annual average mass in the lake	189.1		305.3	
(13) Sulfur turnover time, years[e]	0.82		2.18	

[a]January 8, 1976 to January 27, 1977.
[b]January 27, 1977 to January 11, 1978.
[c]M = mass in lake.
[d]Calculated from "missing SO_4" as explained in the text.
[e]Annual average mass in the lake ÷ total input.

year. For the area of the 9 m plane, this would be 3860 eq of SO_4, about 10% of the "missing sulfate."

INPUT–OUTPUT MASS BALANCES

Sulfur — The sulfuric acid additions increased the sulfate loading to the lake by 7.6 times the natural amount in 1976 and 3.2 times in 1977 (Table 1). The agreement between the calculated increase in SO_4 mass (row 9) with the actual increase in SO_4 mass (row 10) is within the 10% precision of sulfate measurements. The reduction of sulfate to sulfide appears to become an increasingly important part of the sulfur budget of the lake as the SO_4 concentration in the hypolimnion at overturn increases, accounting for 15% of the added sulfur in 1977.

Dissolved inorganic carbon — Inputs of DIC from inflows, direct runoff and precipitation were all very small (Table 2). Losses from the lake by outflow in 1977 were much lower than in 1976, due to the lower DIC content of surface waters. Most of the DIC lost from the lake is presumably caused by degassing of the CO_2 produced by reaction of added hydrogen ion with bicarbonate. In preacidification years the partial pressure of CO_2 in the epilimnion, calculated from DIC and pH, was about 2.5–3 × saturation with the atmosphere. In 1976 and 1977 late summer calculated P_{CO_2}'s were lower, nearly at saturation (Fig. 15). Reasons for this increased degassing efficiency are not

TABLE 2. Dissolved inorganic carbon budgets for Lake 223, 1976 and 1977. Data are in kilograms.

	1976	1977
(1) Inflow stream	45	204
(2) Direct drainage[a]	319	439
(3) Precipitation[b]	0	0
(4) Total input	364	643
(5) Outflow	344	149
(6) Total input − outflow	20	494
(7) Observed ΔM[c]	−134	−1118
(8) Presumed lost to atmosphere (6) − (7)	154	1612

[a]Assumed to be equal in yield/area to the Northwest Subbasin of Rawson Lake (Schindler et al. 1976).
[b]Atmospheric saturation with CO_2 gas is ignored. Bicarbonate content was negligible.
[c]M = mass in lake. Data used are for January 8, 1976, January 27, 1977, and January 11, 1978.

clear. Average wind velocities for 1974–78 for the nearby Rawson Lake station (8 km from the lake) showed no significant differences between years.

Discussion

BICARBONATE IN THE WATER COLUMN AS A BUFFER AGAINST ACIDIFICATION

If only buffering by carbonate equilibria in the water column is considered, the pH and dissolved inorganic carbon in the lake can be related to the quantity of acid added to the lake by performing an ordinary titration calculation using electroneutrality and CO_2 mass balance relationships (Stumm and Morgan 1970). In the treatment that follows the concentrations used will be volume-weighted whole-lake averages.

Prior to acidification the charge balance equation is

$$(1) \quad Alk_o = (HCO_3^-)_o - (H^+)_o$$

where all terms are eq·L^{-1}, the subscript zero repre-

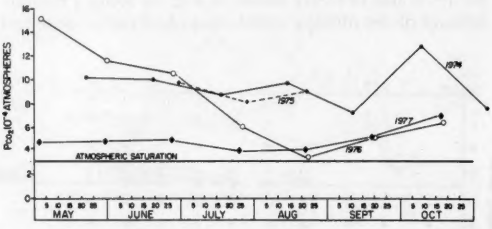

FIG. 15. Partial pressures in the epilimnion of Lake 223 for CO_2 calculated from DIC, pH, and temperature data. The years 1974, 1975, 1976, and 1977. Note that the summer P_{CO_2} decreased in 1976 and 1977, when the lake was acidified.

TABLE 3. Efficiency of acidification at lowering pH and alkalinity in Lake 223 during the first 2 yr of acidification. See text for a description of terms used in this table. All values are volume-weighted whole-lake averages.

Period	Initial conditions			Final conditions			Predicted final conditions		E
	Alk_o $\mu eq \cdot L^{-1}$	pH	DIC $\mu eq \cdot L^{-1}$	Alk_f $\mu eq \cdot L^{-1}$	pH_f	DIC_f $\mu eq \cdot L^{-1}$	Alk_p	pH_p	%
Jan. 1976–Jan. 1977	74	6.65	119	35	6.18	113	−30	4.46	62.5
Jan. 1977–Jan. 1978	35	6.18	113	18	6.05	66	−20	4.60	69.1
Equation 2	71			28			16		22

sents prior to acidification and Alk_o is the carbonate alkalinity. Alk_o is also the difference between the total charge on nonprotolytic cations and the total charge on nonprotolytic anions or

$$(2)\quad Alk_o = (Na^+)_o + (K^+)_o + (Ca^{2+})_o + (Mg^{2+})_o + (Fe^{2+})_o + (Mn^{2+})_o - (Cl^-)_o - (SO_4^{=})_o - (NO_3^-)_o$$

where all terms are $eq \cdot L^{-1}$. Carbonate and OH^- ions are of negligible importance in L223 at both pre- and acidification pHs. The concentrations of H_2S and NH_3 are also negligible.

On acidification an amount of hydrogen ion equal to the increase in SO_4 concentration ($(SO_4^{=})_a$) is added to the lake. The carbonate alkalinity, Alk, then becomes:

$$(3)\quad Alk = Alk_o - (SO_4^{=})_a = HCO_3^- - H^+$$

where all terms are $eq \cdot L^{-1}$ and HCO_3^- and H^+ are the equilibrium values after acidification. Alk can also be defined in a manner analogous to (2) using concentration values after acidification.

The CO_2 mass balance equation for the lake is:

$$(4)\quad DIC = H_2CO_3 + HCO_3^-$$

because carbonate ion ($CO_3^{=}$) is negligible at the observed pH values. We will assume that the DIC does not change upon acidification; only the partitioning between H_2CO_3 and HCO_3^- is a function of pH. This assumption will be examined in detail below. Combining (3) and (4) with the equilibrium expression:

$$(5)\quad K_1 = \frac{[HCO_3^-][H^+]}{[H_2CO_3]}$$

results in:

$$(6)\quad (H^+)^2 + a(H^+) + b = 0$$

where $a = Alk_o - (SO_4^{=})_a + K_1$,

$b = K_1[Alk_o - (SO_4^{=})_a - DIC]$

The ionic strength of L223 is low enough to allow the substitution of concentration for activity in (5). The carbonate alkalinity both prior to and after acidification can also be calculated from:

$$(7)\quad Alk = DIC\left[\frac{K_1}{H^+ + K_1}\right] - H^+$$

using the appropriate values of H^+ and DIC. Equation (7) is readily derived from (1), (4), and (5). The values for K_1 at the lake temperature are interpolated from those of Garrels and Christ (1961).

In our treatment we are using a calculated value of alkalinity for two reasons. We are only looking at the ability of dissolved carbonate species to buffer against pH changes. Precambrian shield lakes are predominantly buffered by the carbonate system. Also, we have only been measuring alkalinity using a standard closed-vessel titration procedure on profile samples since July 1978. Investigations in the future will make use of this measured alkalinity.

By comparing predictions from equation (6) with actual pH changes in the lake, it is possible to assess whether factors other than dissolved carbonate equilibria are influencing the acid neutralizing (buffering) capacity of the lake.

A convenient time period for comparing predicted and actual pH changes is 1 yr, using January data of successive years as the starting and ending points. Shorter time periods, like summer stratification, are difficult to use due to the changing volume of the epilimnion as the thermocline deepens, entraining DIC. The timing and completeness of spring and fall turnover in the lake vary greatly, and January conditions are more consistent from one year to the next.

Using pH, DIC and a volume-weighted average temperature of 2.9°C for January 8, 1976, we predicted that by January 27, 1977 the pH of the lake should have been 4.45 if all of the 203×10^3 eq of added H^+ were buffered by dissolved bicarbonate in the lake water (Table 3). Instead, a pH of 6.11 was observed.

Likewise, beginning with data for January 27, 1977, addition of 107×10^3 eq of H^+ should have resulted in a pH of 4.58 by January 11, 1978. Instead, a pH of 6.05 was recorded. The much higher observed than predicted pHs indicate that there is considerable buffering from factors other than dissolved bicarbonate.

In the above calculation we assumed that the DIC remained constant during acidification (eq (4)). By comparing the initial and final concentrations in Table 3

one sees that the DIC remains nearly constant from January 1976 to January 1977, but decreased from 113 to 66 $\mu eq \cdot L^{-1}$ between January 1977 and January 1978. Using the final DIC value of 66 $\mu eq \cdot L^{-1}$ for 1977 instead of the initial value results in a predicted pH of 4.59, a change of only 0.01 from that above. Therefore equation (6) is insensitive to changes in DIC and our assumption that the DIC remains constant during acidification does not bias our predictions greatly.

A quantitative estimate of the relative importance of any additional buffering capacity can be calculated by comparing initial and final carbonate alkalinities (Alk_o and Alk_f) from equation (7) with the predicted alkalinities from equation (3) (Alk_p). The fraction, E, of acid added which is neutralized by factors other than dissolved bicarbonate is:

$$(8) \quad E = \frac{Alk_f - Alk_p}{Alk_o - Alk_p}$$

If no reaction of acid with species other than dissolved bicarbonate occurs then $Alk_f = Alk_p$ and E is zero. If all of the acid is neutralized by species other than dissolved bicarbonate then $Alk_o = Alk_f$ and E is 1. We shall call this acid neutralization by species other than bicarbonate dissolved in the water column "auxiliary buffering." In 1976, 62.5% of the acid neutralization was accounted for by auxiliary buffering. In 1977 the value was 69.1%.

A comparable alkalinity value should be obtained from E values calculated from equation (2). This was possible only for the second year of acidification, because iron and manganese were not analyzed in January of 1976. For 1977, the value for E is 22%.

This procedure is, however, less satisfactory because it ignores charge-bearing organic molecules, and it is necessary to assume that all of the elements contributing to the calculation are in ionic and nonprotolytic forms. In general, charge-balance calculations on ELA lakes using inorganic constituents alone show the water to be lacking in negative charges (D. W. Schindler unpublished data).

Sources of Buffering

There are a number of biological and chemical processes which could increase a lake's resistance to acidification.

Bicarbonate in sediments could buffer against acidification in the same manner as that in the water column. However, short-term experiments done in 1976 with a number of shallow sediment cores from Lake 223 revealed that sediments in the lake had little capacity for removing hydrogen ion (D. W. Schindler unpublished data). Longer-term investigations are currently under way.

Weak organic acids, i.e. those with pKs higher than sulfuric acid, could also buffer against decreases in pH. Organic acids are not abnormally high in Lake 223. A survey of alkalinity values in 100 lakes of the ELA area indicates that as much as 20% of the alkalinity might be accounted for by such factors. However, determining their contribution to the buffering capacity is difficult due to their complex chemical make-up and unknown ionization constants.

Photosynthesis can also be looked upon as a net consumer of hydrogen ion as in reactions (9) and (10) below:

$$(9) \quad HCO_3^- \rightleftharpoons OH^- + CO_2 \text{ (taken up during photosynthesis)}$$

$$(10) \quad OH^- + H^+ \rightleftharpoons H_2O$$

However, if most of the photosynthesized product is decomposed in the lake, there will be little net buffering over long periods.

Processes which increase the water column concentrations of cations other than H^+ can also be expected to increase the buffering capacity (eq (2)). In lakes with anoxic hypolimnions like Lake 223, release of manganese and iron (II) from sediments may cause the alkalinity to increase. Another way of looking at this is that the DIC generated by the decomposition of allochthonous material (Hesslein et al. 1980; Rau 1978) is stored as bicarbonate in order to balance the charge of the increased iron and manganese. This increases the alkalinity as can be seen from equation (1). The relationship between bicarbonate and dissolved iron in the hypolimnion is almost at the 2:1 ratio necessary for ionic balance up to about 0.25 mmol (Fig. 16). The mechanism of hypolimnetic DIC formation will be discussed below. It should be noted that this buffering by iron mobilization is in effect only during periods of anoxia. As soon as oxygen is mixed into the hypolimnion, Fe^{2+} precipitates as an iron hydroxide, bicarbonate converts to CO_2, and the alkalinity is lost.

Hypolimnetic sulfate reduction during periods of anoxia will increase the buffering capacity by decreasing the total charge on nonprotolytic anions (i.e. $SO_4^=$) (eq (2)). During 1976 the reduction of 7.1×10^3 eq of sulfate increased the volume-weighted average alkalinity by 3.7 $\mu eq \cdot L^{-1}$. This is 10% of the alkalinity present at the end of the year. During 1977 the reduction of 21.5×10^3 eq of sulfate increased the volume-weighted average alkalinity by 11 $\mu eq \cdot L^{-1}$, or 60% of the alkalinity present in January 1978. This calculation assumes that all the sulfate reduced remains precipitated as FeS even after oxygen is mixed into the hypolimnion at circulation. Postcirculation sulfate data (Fig. 2) shows that there is little, if any, increase in $SO_4^=$ after circulation.

From the above, one might speculate that small, deep, protected lakes which develop anoxic hypolimnion offer more resistance to acidification than shallower or more freely circulating lakes of similar alkalinity. While it presently appears that buffering due to sulfate reduction should increase as the sulfate concentration of the lake increases, such predictions must be made with caution, due to the short duration of this experiment. Buffering of this sort has not previously been

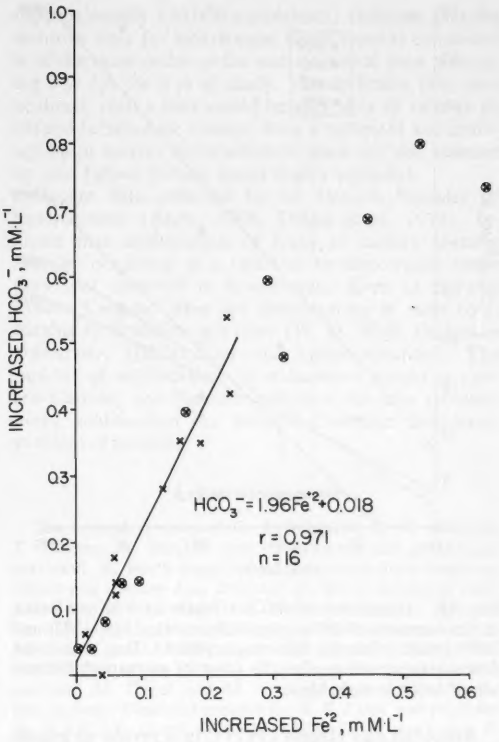

FIG. 16. The molar ratio of the increase in bicarbonate and ferrous ion in Lake 223 deep water during periods of stratification. Data are for 12 and 14 m, and for both summer and winter stagnation periods. Winter data ⊗; Summer data ×.

noted in freshwater. Berner et al. (1970) and Ben-Yaakov (1973) calculated that nearly identical events considerably affected the buffering capacity of pore waters in anoxic marine sediments.

PREDICTION OF THE RESPONSE OF A LAKE TO ACID DEPOSITION

Because so little H^+ and HCO_3^- enter ELA lakes from natural sources, it should be possible to calculate the effect of increasing acidity of precipitation on the carbonate chemistry of the lake. Precipitation at ELA currently has a mean pH of approximately 5. The average amount of precipitation is 0.8 m·yr^{-1}. In a lake with a mean depth of 5 m, this represents an $[H^+]$ addition to the lake of 1.6 μeq·L^{-1}·yr^{-1}. If the efficiency of acid removal of carbonate alkalinity of ~35% observed in Lake 223 is typical, and does not vary with time, this should cause about 0.6 μeq·L^{-1} of HCO_3^- to be lost to the atmosphere assuming that nearly all of the alkalinity is HCO_3^-. This is about 1% of the bicarbonate in an average lake in 1 yr. Bicarbonate concentrations of about 70–100 μeq·L^{-1} in several lakes show no trends over 9 yr of measurement and are presumably in balance with current H^+ loadings. Unfortunately, long-term data are not available for lakes of extremely low buffering capacity (<20 μeq·L^{-1}) in the area.

If the pH of precipitation is reduced to 4, the equivalent $[H^+]$ input is 16 μeq·L^{-1}·yr^{-1}, and HCO_3^- removal of about 6 μeq·L^{-1}·yr^{-1} would be expected. This is about 10% of the mass of HCO_3^- in an average ELA lake per year. There are no obvious compensatory sources of HCO_3^-, and the HCO_3^- content of the system should be depleted in one decade. Good input–output data for more lakes are critically needed to test how far this model can be extrapolated.

Presently, throughout much of eastern Canada and the northeastern U.S.A., pH values of 4 and lower are not unusual in precipitation. It is likely that lakes are presently being affected very rapidly in those regions, a fact confirmed by Dillon et al.'s (1978) summary of the few existing data.

CHANGES IN HYPOLIMNETC DECOMPOSITION DUE TO INCREASED SULFATE

Hypolimnetic DIC in Lake 223 is produced by either oxic or anoxic mechanisms. Oxic generation of DIC is dependent on the completeness of oxygen mixing into the hypolimnion at spring overturn. For example, oxic DIC generation was much lower in 1976 and 1977 than in 1974 and 1978, due to the higher masses of O_2 in the hypolimnion in 1974 and 1978 (Fig. 17). As Fig. 18 shows, there is no evidence so far that acidification has caused decreased total decomposition, as suggested by Hendrey et al. (1976).

DIC is also generated by sulfate reducing bacteria according to Richards (1965):

$$(11)\quad (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 53SO_4^= \rightarrow 106DIC + 53S^= + 16NH_3 + 106H_2O + H_3PO_4$$

With more sulfate reduction occurring as the concentration of sulfate increases, more DIC is generated by this mechanism (Fig. 17).

The observed rate of increase in DIC in the early summer of some years is less than expected from the disappearance of oxygen and sulfate (Fig. 17). Considerable photosynthesis takes place in the hypolimnion during early summer, generating additional oxygen (J. A. Shearer and D. R. DeClercq, Freshwater Institute, Winnipeg, personal communication). While this is probably enough to account for the difference, a calculation is not possible, because we do not know how rapidly photosynthesized material is re-oxidized in the same stratum. Once oxygen disappears, photosynthesis is no longer possible, and the rate of increase in DIC is very close to that expected from oxic and sulfate reduction processes.

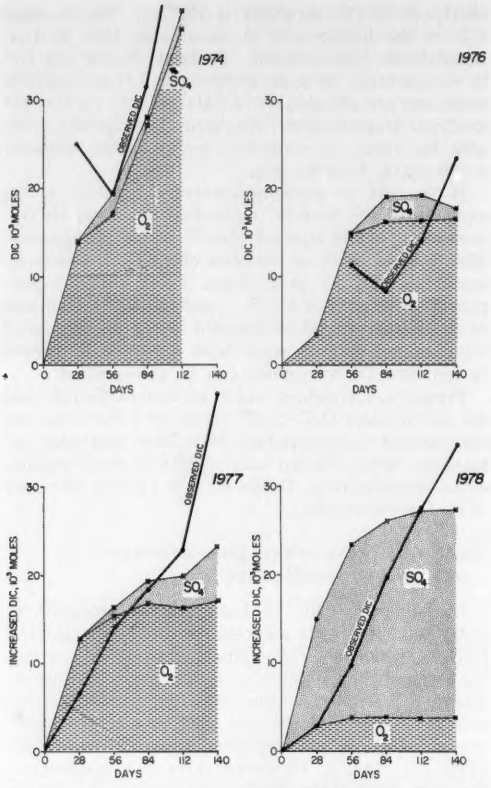

FIG. 17. DIC increases observed in the hypolimnion (below 7 m) of Lake 223 during summer stratification, compared with those expected due to oxic metabolism and sulfate reduction, by making standard metabolic assumptions as described in the text. The greater "expected" than "observed" DIC is assumed to be due to photosynthetic uptake. The difference in oxic decomposition in different years is due to the different degres of spring overturn. DIC generated by sulfate reduction is calculated from equation (11).

DIC continues to increase even after both oxygen and sulfate are depleted, and is presumably generated by methanogenic decomposition. Quantitative measurements of methane generation were not done prior to 1978, but appear to be substantial in that year (C. A. Kelly, Freshwater Institute, Winnipeg, personal communication). There appears to be little increase in methane until late summer, and it is possible that methanogenic bacteria were suppressed by high sulfate concentrations until most of the latter ion was reduced. Winfrey and Zeikus (1977) demonstrated that sulfate inhibited methane production, although the lowest concentration of sulfate which they used was double the maximum concentration in the Lake 223 hypolimnion.

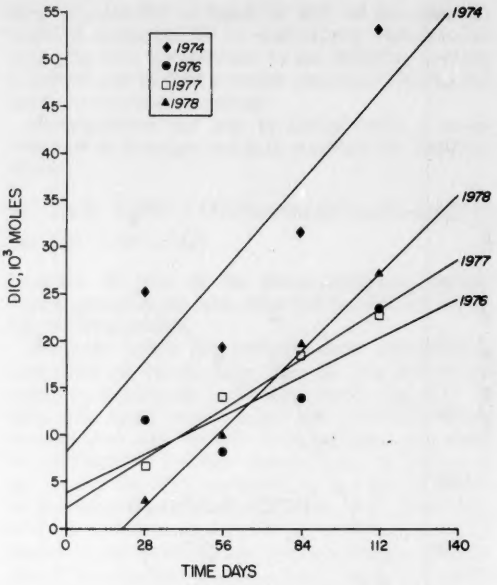

FIG. 18. Generation of DIC at depths of 8 m and below in the summers of 1974 (preacidification), 1976, 1977, and 1978 (years when the lake was acidified). Day zero is the first spring sampling after the onset of permanent thermal stratification in early May.

Reeburgh and Heggie (1977), in a review of anoxic decomposition, observed that sulfate reducers predominated in marine sediments, while methane producers were dominant in fresh waters. Because all of the freshwater lakes where the subject had been studied were of low sulfate concentration, it seems possible that the difference they observed is one between high and low sulfate waters rather than marine and freshwater systems. The observed increase in sulfur reduction in Lake 223 after sulfate concentrations were increased is consistent with this hypothesis. It is possible that acidification with sulfuric acid may change the anoxic metabolism of low sulfate waters such as those in the Precambrian Shield from one dominated by methane producers to one dominated by sulfate reducers.

The fact that H_2S in the hypolimnion is almost always undetectable is expected. Using values of K_1 and K_2 from Ellis and Milestone (1967) and K_{sp} from Berner (1967) only 140 $\mu g \cdot L^{-1}$ of H_2S are required to exceed the solubility product for Fe^{+2} and $S^{=}$ when the Fe^{+2} concentrations is 10 $mg \cdot L^{-1}$ and the pH is 6.8. Iron concentrations are consistently higher than 10 $mg \cdot L^{-1}$ in the deep hypolimnion during summer and winter (Fig. 6).

Comparing the natural input of bicarbonate to Lake 223 with the mass in the lake prior to our experiment

(approximately 150 000 equivalents) indicates that the turnover time for bicarbonate under natural conditions is of the same order as the water renewal time, averaging 8 yr for the 2 yr of study. This indicates that once acidified, such a lake would be very slow to recover its natural bicarbonate content, even if sediment and drainage basin sources of bicarbonate were not also reduced by acid fallout (which seems highly unlikely).

Recent data collected by the Ontario Ministry of Environment (Anon. 1978; Dillon et al. 1978), indicate that acidification of lakes in eastern Ontario may be occurring at a rate two to three times faster than that observed in Scandinavia. Even in extreme eastern Canada, lakes are deteriorating at rates comparable to Scandinavian ones (W. D. Watt, Dalhousie University, Halifax, personal communication). The rapidity of acidification, its widespread nature in eastern Canada, and the dim prospects for lake recovery make acidification the greatest Canadian freshwater problem of all times.

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Effects of Artificial Acidification on the Growth of Periphyton

PETER MÜLLER[1]

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

MÜLLER, P. 1980. Effects of artificial acidification on the growth of periphyton. Can. J. Fish. Aquat. Sci. 37: 355–363.

Acidification caused an increase in periphyton growth in large enclosures in the littoral zone of Lake 223. Diatomeae decreased in relative abundance above $[H^+] = 10^{-6}$ mol/L, and were replaced by filamentous Chlorophyta. The genus *Mougeotia*, dominant in many acidified Scandinavian lakes, was the most tolerant of high acidity. Fewer species and lower species diversity were observed at higher $[H^+]$. Although increases in standing crop were observed, there were no corresponding increases in production. The abundance of invertebrates and microbiota were also unaffected by hydrogen ion concentration.

Key words: lake acidification, periphyton, microcosms, acid rain

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L'acidification a pour effet un accroissement de croissance du périphyton dans de grandes enceintes de la zone littorale du lac 223. L'abondance relative des Diatomeae diminue à $[H^+]$ dépassant 10^{-6} mol/L, lesquelles sont remplacées par des Chlorophyta filamenteux. Le genre *Mougeotia*, dominant dans plusieurs lacs scandinaves acidifiés, est le plus tolérant d'une forte acidité. À $[H^+]$ plus élevé, on observe un nombre moindre d'espèces et une diversité spécifique moindre. Bien qu'on ait observé des augmentations de biomasse, il n'y a pas d'augmentations correspondantes de production. L'abondance des invertébrés et des microbiota n'est pas, elle non plus, affectée par la concentration d'ions hydrogène.

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ONE effect of acid precipitation on Scandinavian streams has been to cause increased growth of periphyton (Hendrey 1976). Despite this observation, no data have been published which document changes in the species composition, standing crop or production of periphyton in acidified lakes, or on the hydrogen ion concentration $[H^+]$ range where changes begin to occur. This study describes changes in periphyton communities which resulted from controlled acid additions to large enclosures in a natural lake.

Materials and Methods

DESCRIPTION OF THE LAKE

Morphometric data for Lake 223 and a morphometric map are shown in Schindler et al. (1980). The lake, located in the Experimental Lakes Area (ELA) in northwestern Ontario, is typical for lakes of this size in the Precambrian Shield. The watershed of the lake is of Precambrian gneisses and granites, forested by typical boreal pine–spruce forest. Brunskill and Schindler (1971) give a more detailed description of the ELA area.

[1]Visiting scientist from the Swiss Federal Institute of Technology, Federal Institute for Water Resources and Water Pollution Control (EAWAG), 1977.

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ACIDIFICATION OF LAKE 223

Electrolyte grade, concentrated sulfuric acid was used for all additions. In the spring of 1977, 6–12 carboys (34 kg each) of acid were added to lower the $[H^+]$ to approximately 10^{-6} mol/L. Additions of acid were made throughout the summer in order to maintain the $[H^+]$ near the 10^{-6} mol/L value (see Schindler et al. 1980 for details on acid additions).

TUBE EXPERIMENTS

Tubes of 10 m diameter and 2–2.5 m depth were constructed as follows: circular wooden rings were sawed from 1″ × 8″ (2.5 × 20 cm) spruce, then nailed and glued together to form a circular ring 3″ (7.5 cm) thick and 100′ (30.5 m) in circumference. These were supported by 6″ × 6″ × 4′ (15 × 15 × 120 cm) styrofoam blocks, banded to the ring in a continuous circle with plastic banding material. The inside of the ring was padded with used fire hose. The tube wall was constructed of 6 mil cross-woven polyethylene, treated to be resistant to ultraviolet light. The material was sewn where necessary with double-lapped seams or fastened with POLY-ZIP® constructed of rigid UV resistant polyethylene. Tubes were anchored with six 10 m lengths of 0.25″ (0.63 cm) cable, attached to concrete anchors which were in turn buried in piles of rocks. Scuba divers sealed the bottom of the tube wall to the sediment by piling a continuous ring of rocks on the material. Schindler et al. (1979) give more details of construction.

TABLE 1. The variation of $[H^+]$ over the time of experiment The $[H^+]$ is given in mol/L.

Tube or lake	Range of $[H^+]$	Representative of $[H^+]$ used as abbreviation in Figures and Tables
E	$10^{-6.4}$–$10^{-6.9}$	$10^{-6.5}$
L223	$10^{-6.8}$–$10^{-6.5}$	$10^{-6.25}$
C	$10^{-6.2}$–$10^{-6.2}$	10^{-6}
B	$10^{-4.3}$–$10^{-5.4}$	10^{-5}
D	$10^{-3.7}$–$10^{-4.7}$	10^{-4}

Tubes were installed in a protected bay near the outlet of Lake 223, in 2–2.5 m of water. The bottom sediment was of medium-grain sand, with an occasional large boulder, submerged log, or ferromanganese deposit.

PERIPHYTON EXPERIMENTS

Four tubes and one lake site were used in the experiment during the summer of 1977. Three tubes, B, C and D, were titrated to $[H^+]$ of 10^{-5}, 10^{-6}, and 10^{-4} mol/L respectively, using H_2SO_4. Periodic additions of acid were made to maintain these approximate concentrations. Tube E was left at its natural $[H^+]$. The range in $[H^+]$ over the time of the experiment is summarized in Table 1.

Light extinction was periodically measured in the four tubes and in the lake as described by Shearer and DeClercq (1977).

Plexiglas plates (10 × 20 cm) were used as substrata for periphyton growth. On May 3, 1977, several of these were laid at the depth of 2 m on the sediment at the bottom of each tube and at one bottom site in the lake between tubes C and D.

To collect a sample, a Scuba diver lifted up the tube wall from the bottom, drew out one plate and inserted it in a plexiglas chamber filled with lakewater. The closed chamber was brought up to the surface. Care was taken to minimize the loss of loosely attached algae. Periphyton was scraped from the surface of the plate with a glass microscope slide and a knife. The plate surface, glass slide, and knife were washed with distilled water, and the sample material was transferred into a plastic bottle. An aliquot of this was preserved in Lugol's solution. Identification and counting were done as described by Kling and Holmgren (1972) and Findlay and Kling (1975). The result is expressed as mg biomass $\cdot m^{-2}$, the biomass being based on measured cell volume. Another aliquot was used for chlorophyll determinations (Stainton et al. 1977).

To get an idea of the error due to loss of loosely attached algae during the sampling procedure, one aliquot of the water in the sampling chamber was sampled for algal counting and another one for measuring chlorophyll. By comparing these with counts of the lake phytoplankton (D. L. Findlay unpublished data) it was possible to estimate the error due to handling procedures. Losses were negligible.

PRIMARY PRODUCTION AND GROWTH

The assimilation of radioactive inorganic carbon (^{14}C) was measured twice in the experiment (June 23 and August 17, 1977), using a light incubator technique (Fee 1973). Equal aliquots of scraped periphyton from each tube were added to bottles of filtered lake water and the primary production in each bottle was measured as outlined by DeClercq et al. (1977). Primary production measured by this method does not represent actual in situ primary production of the periphyton, but gives an idea of the relative production in different tubes.

Algal growth was measured during the period from May 3 to August 17. Samples were taken approximately every second week. On every sampling day three plates from each tube and from the lake were scraped for qualitative and quantitative evaluations. Bacteria in periphyton samples were counted on two days (June 14 and June 23) using the technique described in Hobbie et al. (1977).

CALCULATIONS AND DEFINITIONS OF SYMBOLS

Following Hill (1973) two indices for species diversity relative to the biomass were calculated:

(1) N_0 = total number of identification categories

(2) $N_2 = \dfrac{1}{\sum P_i^2}$

where P_i equals the proportional abundances of the different identification categories in a given sample.

The index of community similarity, S, as described by Whittaker (1972) was calculated:

(3) $S = \dfrac{2C}{A + B}$

where A equals the average number of taxa identified in the control lake, B equals the number of taxa identified in a given tube sample, and C equals the number common to both.

Results

PERIPHYTON BIOMASS

There was no apparent trend in biomass with $[H^+]$. All tubes at $[H^+] > 10^{-6}$ mol/L had average biomasses greater than in the lake ($[H^+] = 10^{-6.25}$ mol/L) or in the tube with the lowest $[H^+]$ ($10^{-6.5}$ mol/L) (Fig. 1).

SPECIES COMPOSITION AND ABUNDANCE

At $[H^+] \leq 10^{-6.25}$ mol/L diatoms dominated the periphyton biomass. Where $[H^+] > 10^{-6}$ mol/L, chlorophyta were dominant. Cyanophyta were less abundant at $[H^+] = 10^{-4}$ and 10^{-5} mol/L than at lower acidities (Fig. 2). At $[H^+] = 10^{-4}$ mol/L there was only one dominant, *Mougeotia* sp A, whereas at lower $[H^+]$, 2–4 dominant species were present

The number of species in all tubes was about the same during the initial colonization period of about 35 d (Fig. 3). Once communities were established, however, it was apparent that fewer species were present at $[H^+]$ of 10^{-5} and 10^{-4} mol/L than in the other tubes. Species disappeared from all three major groups of algae with increased acidity (Fig. 4). More qualitative information about species, cell numbers and biomass for two representative dates is given in Tables 2 and 3.

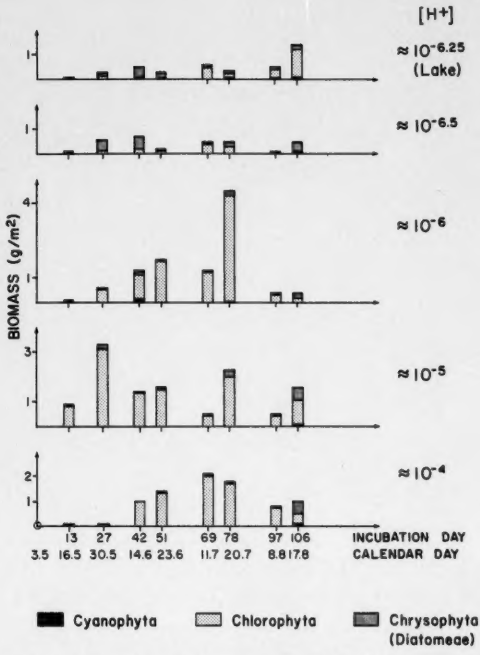

FIG. 1. Biomass of the periphyton for the lake site and four tubes as a function of time; ([H⁺] in mol/L).

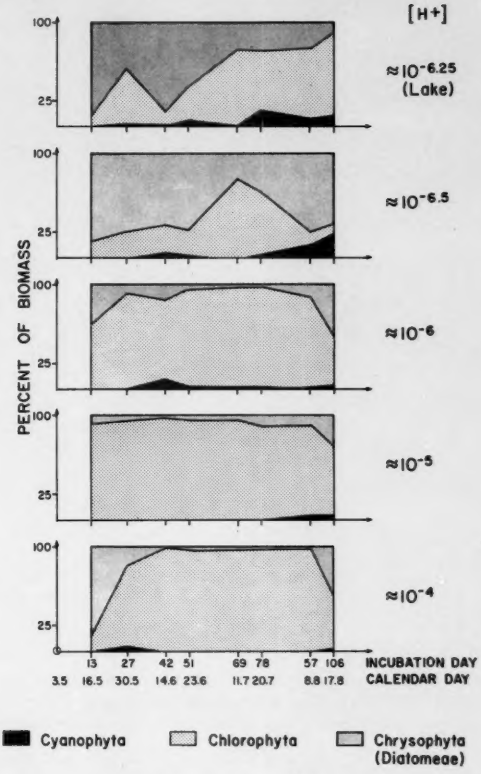

FIG. 2. Percent of biomass of the three main algal groups for the lake site and four tubes as a function of time; ([H⁺] in mol/L).

Species diversity, as indicated by Hill's diversity number N and N_2 decreased with increasing [H⁺], although on August 8 there was an obvious difference between N_0 and N_2 in the [H⁺] range $10^{-6.5}$–$10^{-5.75}$ mol/L (Fig. 5). Community similarity (S) also decreased with increasing [H⁺], except on May 30 where S for $[H^+] = 10^{-4}$, was greater than for $[H^+] = 10^{-5}$ mol/L (Fig. 5).

PRIMARY PRODUCTION

On June 23 the primary production of the periphyton was greater in tube C ($[H^+] = 10^{-6}$ mol/L) than in the three other tubes. Production in the two tubes with the highest [H⁺] (pH 4 and 5) was not significantly different. When production per unit biomass is considered, however, all tubes were very similar, indicating that the higher productions were due largely to a larger biomass (Fig. 6, upper panel).

On August 17, a different pattern was observed. The uptake of carbon was highest in tube B ($[H^+] = 10^{-5}$ mol/L and the production in tube C ($[H^+] = 10^{-6}$ mol/L) was very low. Likewise, there was a great difference in production per unit biomass, atlhough no consistent trend with [H⁺] was observed (Fig. 6, lower panel).

ADDITIONAL EXPERIMENTS

To eliminate the possibility that unique "enclosure effects" were responsible for the observations, on July 20, 1977 some plates with periphyton growth were carefully transferred from tube B ($[H^+] = 10^{-5}$ mol/L and tube D ($[H^+] = 10^{-4}$ mol/L) to the bottom of the control tube ($[H^+] = 10^{-6.5}$ mol/L). After 19 d these plates were sampled together with the regular plates. Both species numbers and diversity of transferred plates rapidly became similar to other plates in their new environment (Table 4), demonstrating that the relationship observed between [H⁺], species diversity and species number was not accidental.

In contrast to the effects of [H⁺], the effects of enclosure in the limnocorrals were insignificant. This is shown by comparing results in the control tube and the lake (Fig. 1–3 and 7):

1) There was no obvious difference in biomass between lake and control tube (Fig. 1).

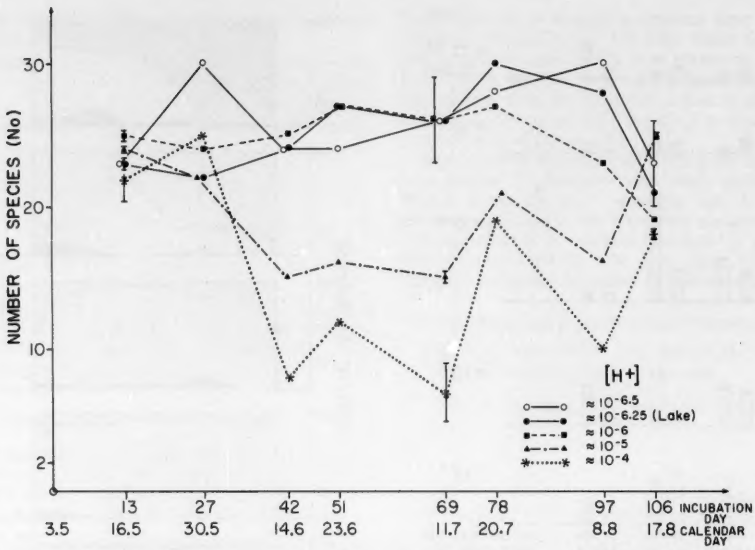

FIG. 3. Number of species for the lake site and four tubes as a function to time. The vertical bars represent the standard deviation of three replicates; ([H⁺] in mol/L).

TABLE 2. Periphyton species*, proportional abundance in terms of biomass and cells for the lake site and the four tubes on May 30, 1977. *Detailed data available on request.

[H⁺] mol/L / May 30, 1977	$10^{-6.5}$		$10^{-6.25}$		10^{-6}		10^{-5}		10^{-4}	
	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)
MAJOR SPECIES										
Chrysophyta (Diatomeae)										
Achnanthes minutissima	27.13	65.70	19.93	48.81	1.57	11.83	0.01	0.32	2.44	7.93
Gomphonema acuminatum	1.15	0.56	3.08	1.51					0.31	0.20
Synedra acus	27.01	16.35	12.97	7.94	2.00	3.77	0.50	3.12	3.56	2.88
Tabellaria fenestrata	9.60	1.39	3.99	0.59	3.40	1.54	0.77	1.15	3.45	0.67
Chlorophyta										
Cosmarium spp.	0.51	1.87			0.92	3.48	0.07	2.57	0.50	1.22
Mougeotia sp. A	1.21	1.47	1.05	1.28	5.84	22.02	48.30	15.04	74.76	36.37
Mougeotia sp. B	18.81	1.74	17.20	1.81	61.11	9.87	34.70	18.51		
Mougeotia sp. C			1.72	0.03			7.03	1.31		
Spyrogyra sp.					3.50	0.27	3.85	0.96		
Zygnema sp.			1.45	0.03						
Uronema sp.	2.64	1.30	29.25	8.48	17.03	32.10	3.07	13.11	0.80	0.64
Cyanophyta										
Pseudanabaena sp.	0.44	3.22			0.14	2.06				
Anabaena sp.			3.40	24.99	0.36	8.15	0.52	38.68	4.26	41.44
OTHER SPECIES										
Chrysophyta	9.60	5.78	5.21	4.43	1.14	1.90	0.53	3.02	7.15	6.85
Chlorophyta	1.90	0.62	0.75	0.14	2.93	3.03	0.65	2.21	2.73	1.80
Total Biomass ($mg \cdot m^{-2}$)	550		284		588		3343		108	
Cells ($10^6 \cdot m^{-2}$)		1514		773		521		895		222

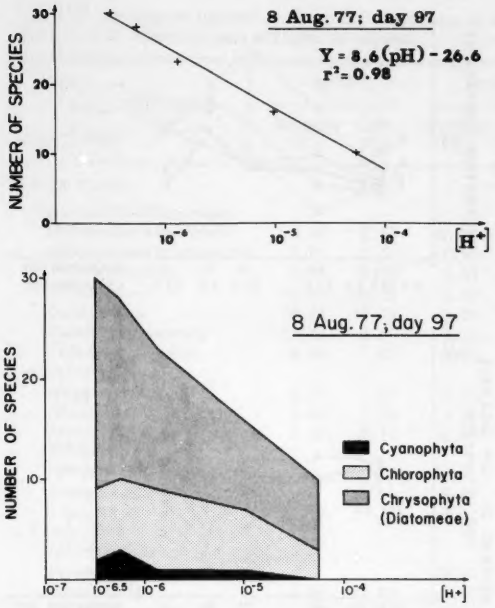

FIG. 4. Effects of hydrogen ion concentration on number of species as shown by regression analysis (*upper panel*) and changes in relative proportions of major groups (*lower panel*) for sampling day Aug. 8, 1977; ([H⁺] in mol/L).

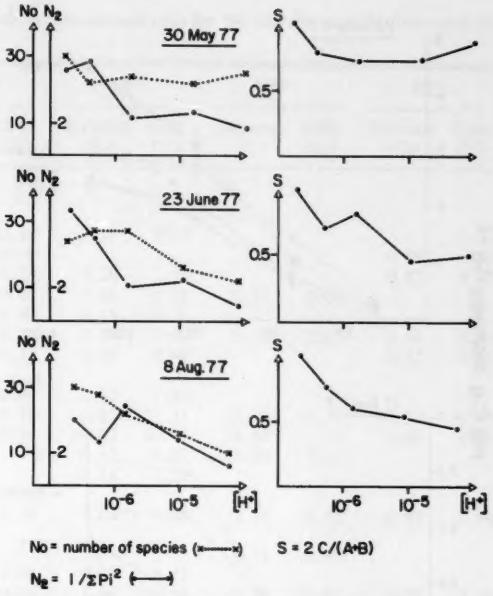

FIG. 5. Effects of hydrogen ion concentration on changes of two indexes of species diversity and on one index of community similarity for three selected sampling days; ([H⁺] in mol/L).

2) The species composition, and the three main algal groups, were similar in both cases (Fig. 2).

3) Diversity index, expressed as number of species, were nearly identical in the lake and the control tube (Fig. 3).

4) Figure 7 shows that both the lake and the control tube had similar light regimes.

Discussion

This study shows that an increase of $[H^+]$ above 10^{-6} mol/L caused more intensive periphyton growth. This agrees with the observations of Hendrey (1976), who observed heavy accumulation of periphytic algae in Norwegian streams affected by acid precipitation. He found greater chlorophyll values and cell numbers at $[H^+] = 10^{-4}$ mol/L than at lower $[H^+]$. Although we did not find any difference in biomass between $[H^+] = 10^{-4}$ and $[H^+] = 10^{-5}$ mol/L each had higher biomasses than at $[H^+] = 10^{-6}$ mol/L.

An increase of $[H^+]$ above 10^{-6} mol/L also caused a significant change in the composition of the three main algae groups (Fig. 2). In the $[H^+]$ range $10^{-6.5}$–$10^{-6.25}$ mol/L, Diatomeae were dominant but this group decreased in biomass with increasing $[H^+]$. Only a few studies dealing with the response of periphyton are published so that few comparisons can be made. Hendrey (1976) found that two algal species (*Mougeotia* sp., *Tabellaria flocculosa*) dominated the periphyton in several streams with high $[H^+]$, whereas in the present study, no Diatomeae species ever dominated the $[H^+]$ range between 10^{-6} and 10^{-4} mol/L. In a field investigation of the effect of acidification on phytoplankton species composition in 115 Swedish lakes, Almer et al. (1974) found that most of the diatom species disappeared above $[H^+] = 10^{-5.8}$ mol/L.

In Lake 223, the increasing dominance by Chlorophyta during the period July 11–Aug. 17 corresponded well with significant rises of $[H^+]$ above $10^{-6.25}$ mol/L; this corresponded with the decreasing importance of Diatomeae and increasing Chlorophyta at higher $[H^+]$ in the tubes. It therefore appears certain at $[H^+] \leq 10^{-6.25}$ mol/L Diatomeae are likely to be favored, whereas the range 10^{-6}–10^{-4} is dominated by Chlorophyta.

Kwiatkowski and Roff (1976) found that in phytoplankton communities of selected lakes of northern Ontario, Chlorophyta diminished in importance while Cyanophyta became dominant (in terms of number of individuals) as acidification proceeded. In contrast, in another study of acidic Ontario Precambrian lakes, Yan and Stokes (1978) found that blue-greens are not usually important contributors to phytoplankton bio-

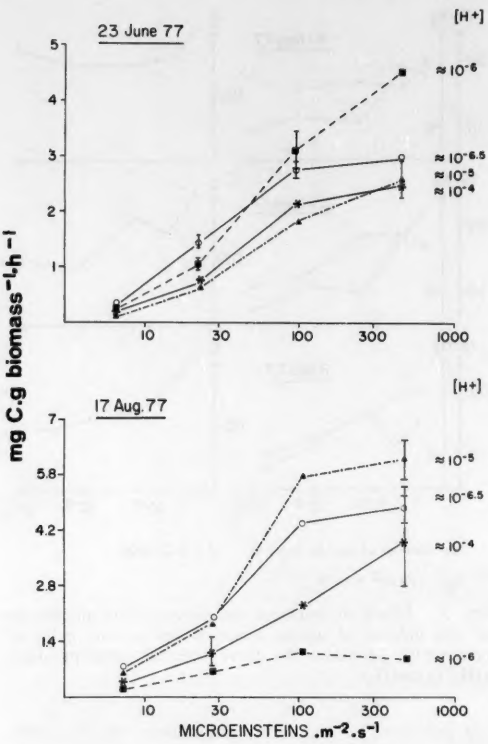

FIG. 6. Plots of photosynthesis vs. irradiance for the 4 tubes shown as production per unit biomass vs. irradiance on two representative dates. The vertical bars join the values obtained for the two replicates at each irradiance; ([H⁺] in mol/L).

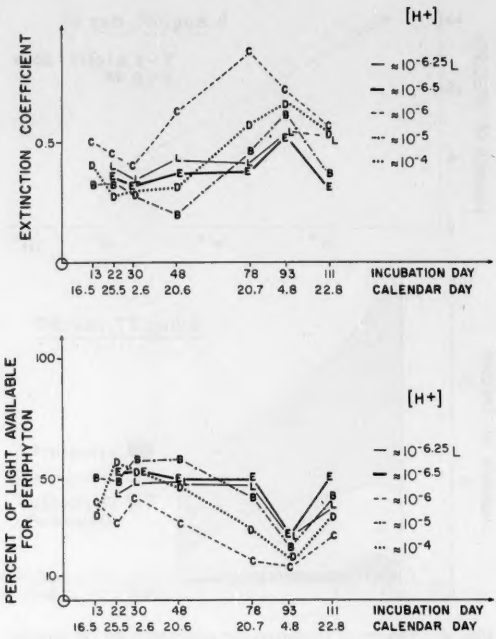

FIG. 7. Plots of light conditions as a function of time for the lake site and the four tubes, shown as extinction coefficient (*upper panel*) and percent of light available (*lower panel*) vs. time; ([H⁺] in mol/L).

mass. The work of Brock (1973) which included benthic algae, also agrees with our results. He found that Cyanophyta were completely absent from habitats with [H⁺] greater than 10^{-4} or 10^{-5} mol/L.

Our observed effect of [H⁺] on number of species in the periphyton community are similar to those found for phytoplankton by several authors (Lind and Campbell 1970; Almer et al. 1974). No author has previously investigated diversity of periphyton under a range of acid conditions. At 10^{-4} mol/L one species, *Mougeotia* sp. A (small cell size) dominated the periphyton, whereas at the lower [H⁺] of 10^{-6} mol/L there were two to four dominant species of filamentous green algae. *Mougeotia* sp. A never dominated the periphyton community in the tube with lower [H⁺].

The two indices of community diversity (N_0, N_2) differed in their sensitivity to [H⁺]. This can be explained by the fact that N_2 is the diversity index most heavily weighted by the more abundant species (Hill 1973) whereas N_0 is independent of proportional abundance. As a result, the decrease in number of dominant species in the [H⁺] range 10^{-6}–10^{-4} mol/L was responsible for the dramatic decrease of N_2 in this range.

The index of community similarity, S, accounts only for presence or absence of species. It was therefore not surprising that S, as a function of [H⁺], responded similarly to N_2. Therefore, because similar numbers of species were found at the end of all tests, S values were similar, even though the species composition obviously changed in tube B, C, and D.

Hendrey (1976) found in his periphyton study, that the accumulation of algae occurred more rapidly at [H⁺] of 10^{-4} mol/L than at lower [H⁺] despite a lower productivity at the higher [H⁺]. In contrast, our experiment on June 23 indicated a more rapidly occurring accumulation of algae at [H⁺] = 10^{-5} and 10^{-4} mol/L than at $10^{-6.5}$ but approximately the same productivity for all [H⁺].

The mechanism by which increasing [H⁺] led to an increased biomass of periphytic algae in artificial stream channels could not be explained clearly by Hendrey (1976). He discussed three possible factors: (1) preference of some species for high [H⁺]; (2) invertebrate grazing; (3) microbiological activity. Since

TABLE 3. Periphyton species*, proportional abundance in terms of biomass and cells for the lake site and the four tubes on June 23, 1977. *Detailed data available on request.

[H⁺] mol/L / June 23, 1977	$10^{-6.5}$		$10^{-6.25}$		10^{-6}		10^{-5}		10^{-4}	
	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)	Biomass (%)	Cells (%)
MAJOR SPECIES										
Chrysophyta (Diatomeae)										
Achnanthes minutissima	0.06	0.17	40.51	60.18	1.17	9.25				
Gomphonema acuminatum	2.71	1.52	0.95	0.28					0.39	0.31
Navicula radiosa	0.69	0.39	6.67	2.29	2.26	3.57			0.83	0.67
Synedra acus	18.60	26.16	1.03	0.57	0.06	0.11	0.11	0.24		
Synedra ulna	20.95	11.78	1.76	0.60	0.18	0.26				
Tabellaria fenestrata	20.89	3.53	2.36	0.21	0.20	0.09	5.31	2.95	0.18	0.04
Tabellaria floculosa	4.44	1.87	0.60	0.13	0.01	0.01			0.02	0.01
Chlorophyta										
Cosmarium spp.	0.93	1.95	0.05	0.05	0.12	1.44				
Mougeotia sp. A	2.68	3.76	3.71	2.75	2.87	11.31	12.76	59.21	96.52	96.40
Mougeotia sp. B	2.28	0.14	10.84	0.34	63.42	10.71	59.00	11.73	0.67	0.06
Mougeotia sp. C					0.42	0.02	18.62	1.30		
Spirogyra sp.					21.18	1.25				
Zygnema sp.			9.27	0.14						
Uronema sp.	13.62	19.15	7.11	5.28	3.04	7.98	1.73	5.31	0.49	0.98
Cyanophyta										
Merismopedia sp.							0.12	3.45		
Pseudanabaeba sp.			1.61	3.58	0.80	5.47				
Anabaena sp.	2.59	21.89	4.57	20.35	1.66	39.19	0.43	11.87	0.03	0.30
OTHER SPECIES										
Chrysophyta	5.61	3.73	8.67	3.17	1.43	4.66	0.42	2.88	0.85	1.23
Chlorophyta	3.94	3.97	0.36	0.04	1.21	4.68	1.50	1.04		
Total Biomass ($mg \cdot m^{-2}$)	232		332		1753		1612		1382	
Cells ($10^6 \cdot m^{-2}$)		550		1490		1483		1157		2308

TABLE 4. Absolute and proportional changes of two indices of species diversity for five treatments, three unchanged and two changed on July 20, 1977. N_0 and N_2 are explained in text. The [H⁺] is given in mol/L.

	Diversity indices					
	Number of species (N_0)		N_2		% Increase or decrease in 19 d	
[H⁺]	July 20, 1977	Aug. 8, 1977	July 20, 1977	Aug. 8, 1977	N_0	N_2
$10^{-6.5} - 10^{-6.5}$ no change	28	30	5.5	4.1	7	−25
$10^{-5} - 10^{-5}$ no change	21	16	2.7	2.9	−24	7
$10^{-4} - 10^{-4}$ no change	19	10	1.2	1.3	−47	8
$10^{-5} \rightarrow 10^{-6.5}$ change	21	34	2.7	2.9	62	7
$10^{-4} \rightarrow 10^{-6.5}$ change	19	30	1.2	5.2	58	330

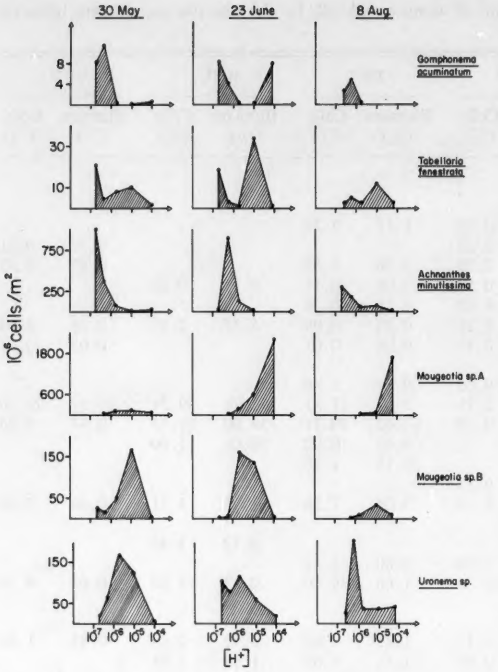

FIG. 8. Effects of hydrogen ion concentration on the total cells per square metre for selected species and selected sampling days; ([H⁺] in mol/L).

he found little evidence for high [H⁺] preference among the 32 algae species observed, he concluded that the reduced rate of algae removal was likely caused by lower activities of invertebrate and micro-heterotrophs. In contrast, this study, which showed similar biomasses at all [H⁺], but different species compositions, could be interpreted as evidence for preference of high [H⁺] among the observed algal species. The effects of [H⁺] on the total cells $\cdot m^{-2}$ for selected species and sampling days are shown in Fig. 8 and Table 5. The three days selected represent the beginning, mid point, and the end of the experiment. It is quite obvious that *Mougeotia* sp. A (small cell size) had its maximum growth near $[H^+] = 10^{-4}$ mol/L, whereas *Uronema* sp. tended to grow better around $[H^+] = 10^{-6}$ mol/L, *Gomphonema acuminatum* did not appear to tolerate the $[H^+]$ of 10^{-6} and 10^{-5} mol/L.

Results obtained from the experiment where plates were traded between tubes confirm this conclusion. *Mougeatia* sp. A, which dominated the community in tube D ($[H^+] = 10^{-4}$ mol/L, disappeared when the community was placed in tube E ($[H^+] = 10^{-6.5}$ mol/L). Also, the growth of *Mougeotia* sp. B, which on July 20 dominated in tube B ($[H^+] = 10^{-5}$ mol/L),

TABLE 5. Species which showed similar distributions like the species presented in Fig. 8.

Species described in Fig. 8	"Similar" species
Gomphonema acuminatum	*Gomphonema ventricosum* *Gomphonema olivaceum*
Tabellaria fenestrata	*Eunotia robusta*
Achnanthes minutissima	*Synedra acus*
Uronema sp.	*Mougeotia* sp. C *Spirogyra* sp.

decreased substantially after the transfer to tube E (control). All species of filamentous Chlorophyta had their maximum growth at [H⁺] between 10^{-6} and 10^{-4} mol/L although different species from this group dominated at different [H⁺]. The increased importance of *Mougeotia* sp. as [H⁺] increased from 10^{-6} to 10^{-4} mol/L corresponded well with observations of Almer et al. (1974), Grahn et al. (1974), and Hendrey (1976).

The number of species of benthic invertebrates in lakes and rivers is reduced as [H⁺] increases (Sutcliffe and Carrick 1973; Hendrey and Wright 1975), and also microbial decomposition is reduced with increasing [H⁺] (Bick and Drews 1973). To get a rough idea of these relationships, in this study, the number of benthic invertebrates was counted once (August 17), and in addition, bacteria in periphyton samples were counted twice. No significant decrease of number of animals with increasing [H⁺] was found. There was also no difference in number of bacteria associated with periphyton. One of the ecological consequences of the simplification of the periphyton community as [H⁺] increases could be a reduction of the variety of food available to benthic grazers. This topic appears worthy of further study.

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Decreased Survival and Calcium Uptake by the Crayfish *Orconectes virilis* in Low pH

D. F. MALLEY

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

MALLEY, D. F. 1980. Decreased survival and calcium uptake by the crayfish *Orconectes virilis* in low pH. Can. J. Fish. Aquat. Sci. 37: 364–372.

As part of a study of the effects of the experimental whole-lake acidification of Lake 223 in the Experimental Lakes Area on the population of the crayfish *Orconectes virilis* physiological responses of adults from this population to low pH were examined in the laboratory. Crayfish survived pH 4.0 for 10 d when they were not moulting but suffered mortality when they were in postmolt stages. Postmolt crayfish held at pH 5.0 for 10 d survived but showed slower progression of molt cycle stages and of calcification of the exoskeleton than individuals held at pH 6.0 or in lake water at about pH 6.7. Uptake of Ca^{++} by postmolt crayfish measured by the use of ^{45}Ca as a tracer was inhibited by pH below 5.75 and ceased altogether below pH 4.0 when these levels of acidity were applied acutely.

Key words: Orconectes virilis, crayfish, molt cycle, mortality, pH, calcification, postmolt calcium uptake, acid rain, lake acidification

MALLEY, D. F. 1980. Decreased survival and calcium uptake by the crayfish *Orconectes virilis* in low pH. Can. J. Fish. Aquat. Sci. 37: 364–372.

Comme partie d'une étude sur les effets de l'acidification expérimentale d'un lac entier dans le lac 223 de la région des lacs expérimentaux sur la population d'écrevisses, *Orconectes virilis*, les réactions d'adultes de cette population à un bas pH ont été étudiées en laboratoire. Les écrevisses survivent à un pH de 4,0 pendant 10 jours si elles ne muent pas, alors qu'aux stades de postmue, elles subissent des mortalités. Les écrevisses d'après mue, maintenues à un pH de 5,0 pendant 10 jours, survivent mais accusent une progression plus lente des stades de leur cycle de mue et de calcification de l'exosquelette comparativement aux individus maintenus à un pH de 6,0 ou dans de l'eau de lac à un pH d'environ 6,7. L'absorption de Ca^{2+} par des écrevisses au stade de postmue, mesurée à l'aide de ^{45}Ca comme marqueur, est restreinte par un pH inférieur à 5,75 et cesse complètement à un pH inférieur à 4,0 quand ces niveaux d'acidité sont appliqués de façon intensive.

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ACIDIFICATION of oligotrophic waters by airborne pollutants, principally SO_2, is a worldwide problem of increasing concern (Likens and Bormann 1974). Generally the effects of increasing acidity on aquatic systems are to decrease population numbers, decrease species diversity, and to favor species known to be acid-tolerant. This has been documented for fish (Beamish and Harvey 1972; Beamish 1974; Wright et al. 1976), phytoplankton (Almer et al. 1974), and for invertebrates (Almer et al. 1974; Sprules 1975; Roff and Kwiatkowski 1977).

The mechanisms by which low pH affects aquatic ecosystems and individual species are not well understood. Therefore, a small, soft-water, oligotrophic lake in the Experimental Lakes Area, Lake 223, is being artificially acidified. As acidification, begun in 1976, progresses at a planned rate of 0.3 pH units per year, numerous chemical and biological parameters are being studied (Schindler et al. 1980).

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This paper describes initial physiological studies on an important species in Lake 223, the crayfish *Orconectes virilis* (Hagen). The purpose is to identify physiological functions sensitive to low pH which may be responsible for or correlated with the failure of the natural population. Population size, dynamics, and fecundity of *O. virilis* in Lake 223 are being determined concurrently to document the pH at which population failure begins and to describe its course (Davies et al. 1977; Schindler et al. 1979).

The first experiments performed determined survival of adult crayfish in static baths at several low pHs to establish the lowest experimental pH which was not lethal. The rates of progression of molt cycle stages and of calcification in these low sublethal pHs were then compared with the rates in unmodified ELA water of pH 6.5–7.0. Following from these experiments and from the suggestion of Borgstrom and Hendrey (1976) that low pH may block Ca^{++} uptake at molt, the effect of low pH on the postmolt uptake of Ca^{++} required for recalcification was measured using ^{45}Ca as a tracer.

Materials and Methods

Site and Methods of Collection of Crayfish

Crayfish were obtained from Lake 223 (L223) located at approximately 49°42′N latitude and 93°42′W longitude in the Experimental Lakes Area (ELA), northwestern Ontario. The lake is 30.2 ha in surface area and 14.4 m in maximum depth. Morphometry, chemical and biological parameters of L223, and the method of acidification are described by Schindler et al. (1980). From early May to October 1976, electrolyte grade sulphuric acid was added to the epilimnion of L223 from the rear of a moving boat. Despite weekly additions of sulphuric acid the pH of the epilimnion dropped by only 0.1–0.2 pH units below 1974 values which were commonly 6.7–7.0. During 1977, the second experimental year, additions of sulphuric acid lowered the epilimnion pH during summer to between 6.0 and 6.2 (Schindler et al. 1980). Continued acid additions during the summer of 1978 resulted in an epilimnion pH between 5.8 and 6.1 from May to June, and between 5.5 and 6.0, usually 5.6–5.9, from late June to September (D. W. Schindler unpublished data). Effect of acid addition on dissolved inorganic carbon (DIC) was pronounced. Beginning in June 1976, DIC in the epilimnion decreased to around 50 $\mu mol \cdot L^{-1}$ from values of 100–200 $\mu mol \cdot L^{-1}$ typical in 1974, 1975, and May 1976 (Schindler et al. 1980). During the summer of 1977, DIC values in the epilimnion were 25–30 $\mu mol \cdot L^{-1}$, and during 1978, around 20 $\mu mol \cdot L^{-1}$ (D. W. Schindler unpublished data).

Crayfish were captured during May and June of 1977 and May to September of 1978, from depths less than 3 m by scuba divers or were obtained incidentally during seining and trapping for fish. They were transported in L223 water to the ELA field station 5 km distant within 6 h of capture.

Maintenance of Crayfish

Crayfish were maintained outdoors at the field station in opaque blue plastic tubs of dimensions 30 cm × 45 cm × 30 cm containing static volumes of about 30 L of water. Tubs were covered with translucent plastic covers to exclude litter and rain, and to allow light to enter.

The laboratory water supply, used for holding crayfish and for all experiments, was drawn from the hypolimnion of Lake 239 (L239). Its average composition during the summers of 1977 and 1978, expressed as the mean ±SE, in $\mu mol \cdot L^{-1}$, was Ca^{++}, 68.6 ± 0.9; Na^{+}, 59.5 ± 3.0; K^{+}, 17.8 ± 0.3; Mg^{++}, 33.3 ± 0.5; $SO_4^{=}$, 48.9 ± 1.9; Cl^{-}, 33.1 ± 0.9; and DIC, 146.3 ± 8.6 ($n = 8$). pH was 6.4–6.9. For comparison, the composition of water taken from the epilimnion of L223 during the summers of 1977 and 1978 averaged Ca^{++}, 59.2 ± 1.5; Na^{+}, 40.6 ± 1.6; K^{+}, 8.0 ± 0.6; Mg^{++}, 21.5 ± 0.7; $SO_4^{=}$, 80.7 ± 2.3; Cl^{-}, 19.4 ± 3.1 ($n = 9$). pH and DIC are described above.

Temperature in the holding tubs was controlled by their placement on a water table cooled by a continuous flow of the hypolimnetic water at 11–13°C. Photoperiod was natural day and night lengths. Plastic flower pots, 7.5 cm in diameter at the top, weighted down with granite stones, were provided as cover. In 1977, crayfish were fed a commercial soft pellet dog food and in 1978, a laboratory-prepared extruded pellet (S. L. Leonhard unpublished data). Food was given two or three times per week and left overnight in the tubs. The water was renewed the morning after feeding. Experiments were carried out between May 5 and July 1, 1977, and between June 8 and September 4, 1978.

Determination of Molt Cycle Stage

Molt stages were identified after Stevenson (1975). His scheme for crayfish is adapted from the well-accepted molt cycle stages for crabs defined by Drach (1939) and are *A* (A_1–A_2), immediately after ecdysis; *B*, in which the carapace usually is leathery; *C* (C_1–C_3), in which the exoskeleton progressively hardens; C_4, intermolt; *D* (D_1–D_4), preparation for molt and *E*, ecdysis. During the intermolt stage, *O. virilis* from ELA does not achieve the degree of hardness exhibited by the same species from Manitoban waters (S. L. Leonhard personal communication). Therefore, postmolt crayfish were staged by subjectively comparing the rigidity of the exoskeleton with the greatest degree of hardness seen in the population. Premolt was staged by observing degree of development of new setae at the edge of the uropods and telson, and by the presence of gastroliths when animals could be sacrificed. Small individuals of less than 2.0 cm carapace length in stage D_3 possessed a soft exoskeleton comparable to *B* stage crayfish and care was required to distinguish between these.

Mortality of Adult Crayfish in Static Baths at Low pH

Adult crayfish were placed in 30-L static volumes of L239 water and maintained outdoors as described above. These baths were adjusted to nominal pHs of 3.0, 4.0, 5.0, and 6.0 with reagent grade H_2SO_4. No buffers were added. Adult crayfish were held in 30 L of unmodified L239 water at initial pH 6.7–7.0 as controls. The pH of the baths except the controls was adjusted the day before an experiment began and monitored and adjusted, if necessary, twice a day during the experiment. The crayfish were transferred to fresh baths at the test pHs every 2–3 d, following feeding. Mortality was noted twice a day. Mortality was defined as lack of abdominal muscle tension as evidenced by failure of the tail to flex when it was extended.

Three experiments were conducted. In the first, temperature was not controlled and crayfish were not fed. In the second and third experiments, crayfish were fed and temperature was controlled.

Rate of Progression of Molt Cycle Stage at Low Sublethal pH

Crayfish at two postmolt stages, *B* and C_1, were placed in baths at nominal pHs 5.0 and 6.0 and in unmodified L239 water at pH 6.7–7.0 for 10 d. The molt stage of each individual was noted at the end of the experiment. In addition, Ca^{++} content of the exoskeleton was determined at the termination of the experiment.

Measurement of Initial Influx of Ca^{++}

Ca^{++} influx was measured by placing crayfish individually into 250-mL beakers containing 10 or 20 μCi (1 Ci = 37 GBq) of ^{45}Ca in 100 mL of L239 water at pH 6.4–6.9 and observing the rate of disappearance of ^{45}Ca from the medium. This rate of disappearance is here referred to as uptake, as distinguished from net uptake, defined later. The disappearance of ^{45}Ca is due to its movement into

the crayfish and not to adsorption to the container or to the crayfish. ^{45}Ca in the medium was measured over time by removing 50-μL aliquots at 30–60-min intervals. The aliquots were added to 15 mL of Amersham PCS fluor and radioactivity was measured by scintillation counting with 80% efficiency. The media were sampled for Ca^{++} analysis at the beginning of the experiment and, in some cases, periodically during the experiment and at the end.

Natural logarithms of CPM of ^{45}Ca (Y) were regressed against time in hours (X) and a straight line $Y = a + bX$ with slope, b, was fitted by the method of least squares. Influx was calculated from the relationships given by Shaw (1959) which reduce to:

$$M = -bA$$

where M is the influx in μmol $Ca^{++} \cdot h^{-1}$. Since the amount of Ca^{++} in the medium containing a postmolt crayfish does not remain constant but declines exponentially, in this study A is defined as the total amount of Ca^{++} in the solution at time 0 in μmoles and the influx calculated is therefore initial influx. Weight-specific influx was calculated by dividing the initial influx by the live (wet) weight of the crayfish in grams and was expressed as μmol $Ca^{++} \cdot g^{-1} \cdot h^{-1}$.

Measurement of Ca^{++} Efflux

To measure Ca^{++} efflux, crayfish were first loaded with ^{45}Ca by placing them individually into 100-mL volumes of L239 water containing 30 μCi for 67 h. Then the crayfish were rinsed in unlabeled water and hemolymph was collected for measurement of specific activity by puncturing the membrane at the base of a periopod. From the hemolymph emerging from the wound, 20 μL was collected for Ca^{++} analysis and 5 μL for ^{45}Ca determination. The wound quickly closed by clotting and the rerinsed crayfish were then each placed in 100 mL of unlabeled L239 water. The appearance of ^{45}Ca in these media were followed by counting 50-μL aliquots of medium at intervals. Efflux was calculated from the initial specific activity of the hemolymph and the rate of appearance of ^{45}Ca in the medium during the 4-h period following the introduction of the crayfish into the unlabeled medium.

^{45}Ca Uptake in Low pH Media

The rate of disappearance of ^{45}Ca from media into individual postmolt crayfish in L239 water of pH 6.4–6.9, termed $b_{control}$, was measured for a 2.5–4.0-h period. Then the pH of the medium was adjusted abruptly with H_2SO_4 to the desired experimental pH, and the rate of disappearance of ^{45}Ca, termed $b_{low\ pH}$, was measured over an additional several hours. The effect of low, experimental pH was expressed as percent inhibition:

$$\left(1 - \frac{b_{low\ pH}}{b_{control}}\right) \times 100\% = \text{percent inhibition}$$

In some cases, after 9 h, low pHs were readjusted upward with KOH to the initial lake water pH range to determine whether the effect of low pH on calcium influx was reversible.

^{45}Ca Uptake in Low HCO_3^- Media

HCO_3^--free artificial lake water was constituted, based on the ionic composition of L239 water, to contain in

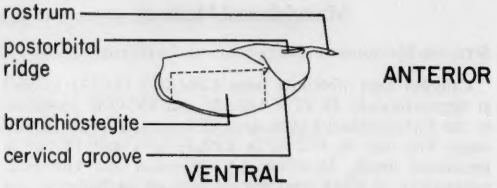

FIG. 1. Lateral view of right side of carapace of *O. virilis*. Broken line shows the section of branchiostegite which was removed for analysis of Ca^{++} content.

μmol $\cdot L^{-1}$, Ca^{++}, 74.9; Na^+, 69.6; K^+, 17.9; Mg^{++}, 35.0; $SO_4^=$, 69.8; and Cl^-, 16.8. Solutions of reagent grade $CaCl_2 \cdot 6H_2O$, KCl, $MgSO_4 \cdot 7H_2O$ and $Na_2SO_4 \cdot 10H_2O$ were prepared such that 1 mL of each plus deionized water to a total of 1000 mL resulted in the above composition. The artificial water was prepared to contain no HCO_3^- and it was bubbled with air from which CO_2 had been removed by passage through a column packed with Ascarite. Measurement of DIC in this artificial water showed it to be low HCO_3 and not HCO_3-free.

The method of measurement of ^{45}Ca uptake in low HCO_3 media was the same as for that in low pH, except that following the measurement of $b_{control}$, the crayfish were transferred to low-HCO_3^- media containing ^{45}Ca.

Determination of Ca^{++} Contents of the Exoskeleton, Hemolymph, and Media

Ca^{++} content of the exoskeleton was measured using the method described by Adegboye et al. (1975). Portions of the branchiostegite were removed as indicated in Fig. 1 and dried for 18–24 h at 100 ± 5°C to obtain dry weights. They were then ashed at 500 ± 50°C for 72 h, dissolved in 0.5 mL of 12 N HCl and brought to 100 mL with deionized water. Hemolymph samples of 20-μL volume were diluted immediately upon collection with 2.0 mL deionized water and frozen until analyzed. Lake water or media, 100 mL in volume, were preserved with 1 mL of 3 N HCl. Concentration of Ca^{++} in all solutions was determined by the chemistry unit of the Freshwater Institute by atomic absorption spectrophotometry (Stainton et al. 1977).

Measurement of pH, DIC, and NH_3 in Media

pH measurements were made with a Radiometer pH meter 29 fitted with a combination electrode. Concentrations of DIC and NH_3 in L239 water or experimental media were measured by the automated methods of Stainton et al. (1977).

Results

Mortality of Adult Crayfish in Static Baths at Low pH

In the first experiment, run from May 12 to 23, 1977, there was no mortality among 15 crayfish, described in Table 1, exposed to nominal pH 4.0, 5.0, and 6.0 (Table 2). All crayfish were hard-shelled. Two females were about to lay eggs and therefore were intermolt. Males were either intermolt, or possibly, premolt. Crayfish of both sexes and a range of sizes

TABLE 1. Data on crayfish used for pH mortality experiments.

Experiment	Total no. of crayfish	Carapace length, cm $\bar{x} \pm$ SE	Weight, g $\bar{x} \pm$ SE	Sex	Interval between capture and start of experiment
1	15	2.15±0.05	2.76±0.18	7 f 8 m Form I	1 or 2 d
2	60	2.35±0.04	—	f:m, 3:4	0.5–1 d
3	96	2.38±0.05	—	f:m, 1:2	1–4 d

TABLE 2. Experimental conditions and results of the pH mortality experiments.

Experiment	Experimental period	Range of temperature recorded (°C)	Nominal pH	Range of pH recorded	Initial no. of crayfish	Average daily mortality as % of initial no.
1	May 12–23, 1977 251 h	15–25	4.0	4.00–4.30	5	0
			5.0	5.00–5.50	5	0
			6.0	5.95–6.40	5	0
2	June 1–4, 1977 67 h	11–16.8	3.0	2.95–3.10	11	14.2
			4.0	3.95–4.20	11	14.7
			5.0	4.90–5.15	11	0
			6.0	5.60–6.10	9	2.9
			L239 water	5.85–6.70	18	2.9
3	June 8–28, 1977 470 h	10–16.5 from day 1–15; 17.5–21.5 from day 16–20	3.0	2.85–3.20 (2.6 once)	16	3.6
			4.0	3.80–4.25	16	1.9
			5.0	4.75–5.40	16	1.0
			6.0	5.78–6.38	16	0.7
			L239 water	6.00–7.05	16	0.1

had been placed in each of the three static baths. pH of baths typically rose with time and was adjusted twice daily back to the nominal pH. Ranges of recorded pH are shown in Table 2. Temperature of the baths was not controlled.

By the time the second experiment was conducted, from June 1 to 4, 1977, many individuals in the population in L223 were molting. Sixty crayfish were distributed among the pH baths so that each contained a range of sizes; crayfish were in the approximate ratios 4 males: 2 females without eggs: 1 female carrying eggs and included a variety of molt cycle stages (A_2 to C_4). Mortality occurred in the pH baths at the following average daily rates, as % of the initial number in the bath: pH 3.0, 14.2%; pH 4.0, 14.7%; pH 6.0 and unmodified L239 water, 2.9%. In nominal pH 5.0, there was no mortality (Table 2). Temperature was controlled within a narrower range than for the first experiment.

Data on the molt stage of the animals which died at pH 3.0 and 4.0 in the second experiment indicated that crayfish in early postmolt stages were more likely to die than other stages, although there was considerable individual variability. The four crayfish which died at pH 3.0 were molt stages A_2, early *B*, *B* and late *B* whereas the seven survivors at 67 h were A_2, C_1, two C_2, and three intermolt. At pH 4.0, crayfish which died during the experiment were early A_2, late A_2, *B*, and one intermolt, whereas the survivors were early *B*, *B*, two late *B*, and two intermolt.

The third experiment was carried on from June 8 to 28, 1977, after the peak molting period in L223, and all males were Form II. The adult females had recently released young but most had not yet molted. The crayfish were placed in the tubs so that each contained both sexes and a range of sizes. They were not individually categorized to molt stage but the predominant molt stage overall was C_2. Average daily mortality was greatest at nominal pH 3.0, 3.6%, and less at pH 4.0, 1.9%. At pH 5.0 and 6.0, mortality was 1.0 and 0.7% and in unmodified L239 water, 0.1%. Rates of mortality were lower than during the second experiment. This is attributed to the smaller proportion of crayfish in *A* and *B* stages among the experimental animals.

RATE OF PROGRESSION OF MOLT CYCLE STAGES AT LOW SUBLETHAL pH

Following from the observation that low pH was more likely to be lethal for early postmolt crayfish than for those in later postmolt or intermolt stages, possible sublethal effects of pH 5.0 and 6.0 on rate of progres-

sion of molt cycle stages and on calcification were studied. The crayfish were captured from L223 either 0.5 or 2 d before the start of the experiment. The experiment was conducted for a 229-h period from June 6 to 16, 1977 beginning with 24 *B* stage crayfish of mean carapace length, 2.14 ± 0.09 cm, distributed in equal numbers between pH 5.0, 6.0, and L239 water baths, and 17 C_1 crayfish of mean carapace length, 2.40 ± 0.11 cm, similarly distributed. There was no mortality during the experiment.

Crayfish at pH 5.0 progressed more slowly to later molt stages than crayfish at pH 6.0 or in L239 water. After 229 h, 6 of 8 *B* stage crayfish in L239 water, and 7 of 8 at pH 6.0 had progressed to stage C_1 or beyond, whereas only 3 of 8 at pH 5.0 did so. All 5 C_1 in L239 water and 5 of 6 at pH 6.0 progressed to at least stage C_3. One crayfish molted in the lake water. At pH 5.0, on the other hand, only 2 of 6 C_1 crayfish progressed to C_3. The pH range recorded for pH bath 5.0 was 4.85–5.35; for bath 6.0, 5.80–6.16 and for L239 water, 6.55–6.95. Temperature ranged from 10.5 to 14.5°C.

To measure the effect of bath pH on the rate of calcification of the postmolt exoskeleton, the Ca^{++} content as percentage dry weight of a section of branchiostegite was compared at the end of the experiment among the *B* stage crayfish from the three baths and similarly among the C_1 stage crayfish. It was assumed that the average Ca^{++} content at the beginning of the experiment was uniform among the three groups of *B* stage crayfish and among the groups of C_1 crayfish. After 229 h, Ca^{++} content of the exoskeleton as percentage dry weight was not statistically different among the groups of *B* stage crayfish exposed to the three pH baths, being ($\bar{X}$ ± SE), 10.6 ± 1.0 at pH 5.0, 11.5 ± 0.9 at pH 6.0, and 10.4 ± 1.1 in L239 water. In contrast, the exoskeletons of the C_1 crayfish, excluding the individual which molted, by the end of the experiment contained the following Ca^{++} as percentage dry weight: 11.7 ± 1.1 at pH 5.0; 13.6 ± 1.1 at 6.0, and 15.7 ± 0.8 in L239 water. Final Ca^{++} content in the C_1 crayfish at pH 5.0 was significantly lower than that from L239 water ($P < 0.05$) although the other differences were not significant. This is evidence that pH 5.0 retards calcification in this species.

The apparent lack of effect of pH on *B* stage crayfish can be explained by reference to Table 3 which shows that Ca^{++} as percentage dry weight does not change over the *B* and C_1 molt cycle stages. Travis (1965) reports that during this portion of the molt cycle, Ca^{++} and the organic matrix are being deposited concomitantly. Thus for the *B* stage crayfish, Ca^{++} in mg·cm^{-2} surface area is a more useful measure of calcification than Ca^{++} as percentage dry weight. Data in Table 3 are from 115 crayfish including those from the pH baths 5.0, 6.0, and L239 water used in the mortality experiments and from other individuals held in L239 water for up to 2 wk, and exclude the individuals from the present experiment.

TABLE 3. Ca^{++} content as % dry weight of the exoskeleton of *O. virilis* over the molt cycle determined by analysis of a section of branchiostegite.

Molt cycle stage	Ca^{++} content as % dry weight ± SE	No. of crayfish
A_2	7.1±2.0	3
B	11.8±0.7	11
C_1	11.7±0.5	42
C_2	14.8±0.5	20
C_3/C^4	16.8±0.3	39

Ca^{++} Influx, Efflux, and Net Uptake

Calcification of the new exoskeleton during the postmolt period requires net accumulation of Ca^{++} from the environment (Greenaway 1974b). Accumulation, termed net uptake, is the difference between influx and efflux, both measured by radioactive tracers. Influx was measured in crayfish at various stages of the molt cycle but efflux was measured only during postmolt to confirm that Ca^{++} was accumulated during that period.

Values of initial influx of Ca^{++} for 35 crayfish in 1977 and 118 crayfish in 1978 are given in Table 4. Ca^{++} influx was dependent upon the stage of the molt cycle. It was greatest immediately following the molt, i.e. in *A* stages, declined during *B* and early *C* stages and fell to relatively very low values during C_3, C_4, and *D* stages. A crayfish included in Table 4 (1977) in molt stages *D* and A_1 underwent ecdysis during the measurement of Ca^{++} uptake. Prior to ecdysis (D_4), the Ca^{++} influx was 0.02 μmol·g^{-1}·h^{-1}, whereas in the several hours immediately following molting influx was 2.26 μmol·g^{-1}·h^{-1}. Each crayfish reported in Table 4 was used only once except for the latter case. During 1977, mean carapace length of the crayfish was 2.39 ± 0.06 cm and mean weight, 3.33 ± 0.25 g whereas in 1978 mean carapace length was 2.46 ± 0.02 cm and mean weight, 3.70 ± 0.10 g.

Data from the 2 yr are reported separately because the lake from which the crayfish were collected, L223, differed in pH and DIC concentration from 1977 to 1978. The values of initial influx obtained in 1978 were

TABLE 4. Initial Ca^{++} influx into crayfish at various stages of the molt cycle determined in the summers of two years. Number of crayfish is in parentheses.

Molt cycle stage	Mean initial influx as μmol Ca^{++}·g^{-1}·h^{-1} ± SE	
	1977	1978
A_1	2.26 (1)	0.42±0.04 (3)
A_2	1.33±0.17 (3)	0.51±0.08 (9)
B	0.90±0.09 (9)	0.38±0.04 (43)
C_1	0.77±0.11 (14)	0.16±0.02 (40)
C_2	0.57±0.10 (2)	0.19±0.04 (15)
C_3/C_4	0.002±0.00 (2)	0.04±0.01 (7)
D	0.05±0.03 (4)	0.03 (1)

lower than those measured in 1977 for comparable stages of the molt cycle. The crayfish used in 1978 were on the average larger than those used in 1977, but this factor cannot explain the difference. In neither 1977 nor 1978 was there a relationship between body weight and initial influx for *B* stage crayfish. In 1977 temperatures during three periods of measurement of influx were 15 ± 2.3°C, 12.5 ± 1.0°C, and 13 ± 1.0°C. During 1978 temperature was more constant (±0.3 to ±1.0°C) during an experiment due to placement of the beakers on a flow-through water table. Since the cooling water for the table was drawn from the hypolimnion of L239, it was 9°C in June and rose to 16°C by August. There was no statistical relationship between temperature during the period of measurement and initial influx for *B* stage crayfish in 1978 ($n = 43$). Since the temperature range was similar during 1977 and 1978 and temperature did not influence influx in 1978, differing temperature does not appear to explain the 1977–78 differences in initial influx for the same molt stages.

The possibility that the lower influxes measured in 1978 were due to physiological adjustments by the population in L223 to increasing acid stress will not be considered until data from further years can be examined.

Efflux of Ca^{++} measured in seven postmolt crayfish in 1977 averaged 0.014 ± 0.004 $\mu mol \cdot g^{-1}$ wet weight$\cdot h^{-1}$ ($\bar{x} \pm$ SE). These values were 2.00 ± 0.67% of the initial influxes measured in the same individuals 3 d previously. The crayfish were in molt stages A_2, *B*, C_1, and C_2. The individual at A_2 demonstrated no loss of ^{45}Ca, i.e. zero efflux. Specific activity of the hemolymph of the crayfish following the 6-h period in the loading media, which initially contained 30 μCi of ^{45}Ca, was 4.04 DPM ^{45}Ca per μmol Ca^{++}. Consistent with high influx relative to efflux by postmolt crayfish is the observation that the DPM of ^{45}Ca of the loading media was reduced to a mean of 0.8% of the initial DPM by the end of the 67-h period. Therefore, during postmolt virtually all of the Ca^{++} taken up by the crayfish is retained.

Effect of Low pH on Ca^{++} Uptake by Postmolt Crayfish

The rate at which ^{45}Ca disappears from the medium, i.e. b_{normal}, multiplied by the initial amount of Ca^{++} in the medium (A) is initial influx. Thus factors which affect b will affect influx and net uptake, unless efflux also changes. For simplicity, effects on influx have been studied here by measuring changes in b in low pH.

The effect of lowered pH on Ca^{++} uptake is shown in Fig. 2 for crayfish studied in 1977 and 1978. 0% inhibition indicates that $b_{low\ pH}$, the slope of the line relating ln CPM to time in low pH conditions, was the same as b_{normal}, the slope during the initial several hours of the experiment. For the determination of b_{normal}, the pH of the medium began between 6.0 and

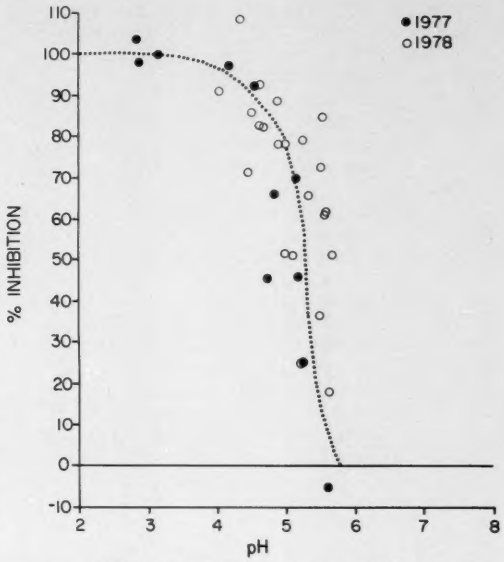

FIG. 2. Progressive inhibition of Ca^{++} uptake in *O. virilis* by low pH. Percent inhibition is plotted against the mean pH of the medium during the period of measurement. Data from two years 1977 and 1978 are shown.

6.7. Figure 2 shows that when the pH of the medium was adjusted to below 5.75, Ca^{++} uptake was partially inhibited. At about pH 4.0, Ca^{++} uptake was completely inhibited, i.e. $b_{low\ pH}$ was zero. Inhibition was at least partially reversible by raising pH to between 6.0 and 7.0. In six crayfish studied in 1977, inhibition was reversed, although the rate of uptake did not fully return to its initial rate when it had been strongly inhibited. More work is required to establish the degree of reversibility.

Changes in pH, DIC, and NH_3 in the Media During Ca^{++} Uptake

The pH and DIC content of media were followed during Ca^{++} uptake in unmodified L239 water to gain possible information on ionic exchanges accompanying Ca^{++} uptake. This could be useful in testing hypotheses to explain the effects of low pH on Ca^{++} uptake.

The pH of media containing crayfish fell abruptly during the first 0.5–2 h of measurement of b_{normal}, by as much as 0.8 pH units ($n = 6$ in 1977; 36 in 1978). Thereafter, pH leveled off or slowly rose. The maximum differences in $[H^+]$ in $\mu eq \cdot L^{-1}$ (Y) recorded between media with crayfish and the respective control media without crayfish at identical times were regressed against b_{normal} (X) for each crayfish. The greater the rate of calcium uptake, the greater was the maximum

difference in $[H^+]$. The least squares fit of the points was $Y = 0.33 + 0.73X$ ($r = 0.72$; probability that $b = 0$ was <0.01). Time at which the maximum difference was recorded varied from 0.5 to 4.0 h. Thus, it appears that Ca^{++} uptake is associated with an increase in $[H^+]$ external to the animals. The appreciable value of the Y-intercept indicates that the $[H^+]$ increases externally to some extent even for crayfish not taking up Ca^{++}.

DIC increased in the media of crayfish, presumably due to the excretion of metabolic CO_2. The average increase in the 100-mL media of nine crayfish was 1.11 $\pm$ 0.05 μmol DIC$\cdot g^{-1}$ live weight$\cdot h^{-1}$. There was no relationship between b_{normal} and rate of DIC increase.

These media were open to the air possibly allowing CO_2 to escape from the surface. Therefore, in a separate experiment, 125-mL volumes of media containing a crayfish were closed to the air and rates of DIC production were measured. After 3 h, the DIC in media containing crayfish increased by 1.25 μmol DIC$\cdot g^{-1}$ live weight$\cdot h^{-1}$ ($n = 12$).

As a possible cause of the leveling or slow increase of pH observed in media containing crayfish, the waste product NH_3 was monitored. In the 125-mL volumes of media closed to the air, NH_3 increased by 0.131 $\pm$ 0.014 μmol$\cdot g^{-1}$ live weight$\cdot h^{-1}$ ($n = 12$) based on a 3-h period of measurement. Initial concentrations of NH_3 in the media were 1.435 $\pm$ μmol $NH_3 \cdot L^{-1}$.

Effect of Low-HCO_3^- Media on Ca^{++} Uptake

Experiments were carried out to examine the possibility that the inhibition of Ca^{++} uptake by low pH is due to the unavailability of HCO_3^-, due to shifting of the equilibria between the chemical forms of DIC away from HCO_3^-. Greenaway (1974b) suggests that 60% of the net uptake of Ca^{++} during postmolt requires accompanying HCO_3^- for electrial balance.

Attempts to prepare HCO_3^--free artificial lake water were not totally successful even when the water was bubbled with CO_2-free air. It contained from 5 to 24 μmol DIC$\cdot L^{-1}$ ($n = 6$), presumably due to invasion of CO_2, compared with L239 water with 139–176 μmol DIC$\cdot L^{-1}$ ($n = 6$).

Rate of Ca^{++} uptake in the low-HCO_3^- media expressed as percentage inhibition for four crayfish (2.43 $\pm$ 0.11 cm carapace length; 3.49 $\pm$ 0.58 g) was 20.1, 5.8, −37.8, and −49.5%. The mean −15.4 $\pm$ 16.8% is not different from 0%. There was no inhibition of Ca^{++} uptake by exposure to the low-HCO_3^- medium.

Increase in DIC in the media of crayfish in low-HCO_3^- media closed to the air was compared with DIC increase in L239 water. After 3 h, crayfish in the low-HCO_3^- media increased the DIC of the media by 1.75 μmol$\cdot g^{-1}$ live weight$\cdot h^{-1}$ ($n = 11$). This is a significantly greater ($P < 0.01$) increase than for the crayfish in L239 water of 1.25 μmol DIC$\cdot g^{-1} \cdot h^{-1}$ given above.

Discussion

Little is known about the mechanisms which cause the failure of invertebrate populations in acidic waters. Borgstrom and Hendrey (1976) speculate that the effect of low pH on crustaceans may be to block the Ca^{++} uptake mechanism at molt. They found that pH of 5.0 and below caused high mortality in adults of the amphipod *Gammarus lacustris* G. O. Sars and that at pH of 5.5 and below early life stages of the branchipod *Lepidurus arcticus* (Pallas) either did not survive or were delayed in molting to the next stage. Shaw (1960) reported that pH below 6.0 inhibits the absorption of Na^+ by the crayfish *Astacus pallipes* Lereboullet.

In the present study crayfish in early postmolt stages were more likely to die in nominal pH 3.0 or 4.0 than those in later postmolt or intermolt stages. Postmolt crayfish survived exposure to nominal pH 5.0 for 10 d but progressed through the molt cycle stages and calcified more slowly than at pH 6.0 or 6.7. These results and the direct evidence of inhibition of ^{45}Ca uptake during postmolt by low pH are consistent with the above hypothesis of Borgstrom and Hendrey (1976).

Postmolt crayfish depend upon uptake of Ca^{++} from the aquatic environment to calcify the new exoskeleton. Although some Ca^{++} is stored as gastroliths or bound to blood proteins by premolt *O. virilis*, substantial quantities of Ca^{++} are excreted to the environment (McWhinnie 1962). Adegboye et al. (1975) consider that the Ca^{++} stored in the gastroliths is insufficient in quantity for calcification of the new exoskeleton. Food may also supply a portion of the Ca^{++}.

Calcium balance over the molt cycle has been studied extensively in the crayfish *Austropotamobius pallipes* (Lereboullet) by Greenaway (1972, 1974a, b). In that species, net uptake of Ca^{++} begins 15–30 min after molt and is immediately at a high rate of about 2.5 μmol $Ca^{++} \cdot g^{-1} \cdot h^{-1}$. During most of stages *A* and *B*, net uptake rate is about 2 μmol$\cdot g^{-1} \cdot h^{-1}$ and it falls between stages B_2 and C_1 to about 0.2 μmol$\cdot g^{-1} \cdot h^{-1}$. The results reported here for *O. virilis* differ from those of Greenaway in the lower values for Ca^{++} influx in postmolt stages *A* and *B* in both years (1977 and 1978) and in the lack of a sharp drop in Ca^{++} influx between *B* and C_1 stages. The lower rates at which *O. virilis* from ELA waters takes up Ca^{++} may be related to the extremely low Ca^{++} levels (60 μmol/L Ca^{++}) compared with the water used by Greenaway (1000 μmol/L Ca^{++}). The lack of a sharp drop may indicate that Ca^{++} uptake requires a larger portion of the molt cycle for completion in the soft ELA waters. *Orconectes virilis* obtained from the Rat River, Manitoba (1760 μmol/L Ca^{++}) and tested in that water during postmolt stages had initial influx rates of 2.3–5.5 μmol$\cdot g^{-1} \cdot h^{-1}$ ($n = 7$) (S. L. Leonhard unpublished data), but the pattern of Ca^{++}uptake over the molt cycle is still under study.

Greenaway (1974b) proposed a scheme of ionic movements during postmolt in which 60% of the net uptake of Ca^{++} is accompanied by HCO_3^- for electrical balance and 40% is exchanged for H^+. The HCO_3^- would be deposited together with the Ca^{++} and $CaCO_3$ during calcification. A second source of bicarbonate for calcification may be metabolic CO_2 which would yield excess H^+. H^+ is further released during the precipitation of Ca^{++} and HCO_3^- (Simkiss 1976). The evidence on which Greenaway bases this scheme is the 60% reduction of net uptake of Ca^{++} by postmolt *A. pallipes* in solutions without HCO_3^- and by its return to full rate when HCO_3^- is added to the solution. The method which the latter author used to prepare HCO_3^--free water and maintain it HCO_3^--free during the experiment was not sufficiently described to be repeated here.

Although there is not a firm understanding of the mechanism of Ca^{++} uptake by *O. virilis* in soft waters, the inhibitory effect of low pH on postmolt Ca^{++} uptake in this species is possibly by one or more of the following means: 1) the scarcity of HCO_3^- to accompany Ca^{++} for electrical neutrality, 2) the high concentration of H^+ in the external medium which may interfere with the exchange of internal H^+ for external Ca^{++}, 3) direct effects of H^+ on the active transport system such as by altering molecular configuration, or 4) the effect of $SO_4^=$, from the H_2SO_4 used for acidifying, on the active transport system.

It is unlikely that $SO_4^=$ was in sufficiently high concentration in the low pH media to be inhibitory. In the static pH baths during the mortality studies it varied from about 45 to 575 $\mu mol \cdot L^{-1}$ depending upon the cumulative amount of H_2SO_4 which had been added. *Orconectes virilis* inhabits prairie waters such as the Souris River, Manitoba which varies in the range from 177 to 5310 $\mu mol \cdot L^{-1}$ (median, 1730 $\mu mol \cdot L^{-1}$) $SO_4^=$ (Canada, IWD 1978).

The increase in $[H^+]$ in the media of *O. virilis* and the fact that the greater the rate of Ca^{++} uptake, the greater the increase in $[H^+]$ could be explained in two ways. H^+ could be released to the outside in exchange for Ca^{++} as suggested by Greenaway. This would provide for the elimination of the H^+ produced by Ca^{++} precipitation. But equivalents of Ca^{++} taken up by *O. virilis* were always many times greater than the net equivalents of H^+ appearing in the medium. An alternative hypothesis is that the H^+ results from the excretion of CO_2, giving rise to H^+ and HCO_3^-, followed by an uptake of HCO_3^- together wth Ca^{++}. This would leave excess H^+ in the external medium. The fact that the DIC increased in the media of crayfish, not decreased as expected by this hypothesis, would be explained if CO_2 excretion exceeded the HCO_3^- needs.

Requirement for HCO_3^- to accompany Ca^{++} for electrical balance would explain the close correspondence between Fig. 2 which represents inhibition of

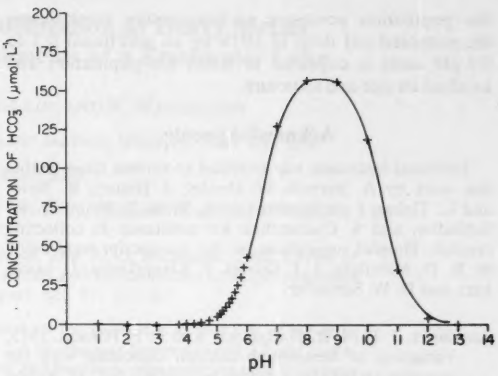

FIG. 3. Calculated concentration of HCO_3^- as a function of pH for water at 15°C containing 162 $\mu mol \cdot L^{-1}$ DIC.

Ca^{++} uptake by low pH and Fig. 3 which indicates the availability of HCO_3^-. The latter figure shows the calculated concentration of HCO_3^- in L239 water with total DIC of 162 $\mu mol \cdot L^{-1}$ at 15°C as function of pH. At pH 6.0 the amount of HCO_3^- expected in 100 mL of medium was 4.38 μmol compared with an initial amount of Ca^{++} of approximately 6.5 μmol. At pH 5.5 HCO_3^- was reduced to 1.70 μmol, to 0.58 at pH 5.0 and to 0.12 μmol at pH 4.5 and may progressively limit Ca^{++} uptake.

Further experimentation is required to distinguish between the hypothesis that low pH inhibits Ca^{++} uptake by shifting the form of the DIC away from the required HCO_3^- (1 above) and the hypothesis that the high external H^+ inhibits the release of H^+ exchanged for Ca^{++} or affects the active transport system directly (2 and 3 above). A further hypothesis is that more than one mechanism operates.

Although low pH inhibits postmolt Ca^{++} uptake it is unlikely that this is the direct cause of death. As mentioned above, pH below 6.0 inhibits the absorption of Na^+ by *Astacus pallipes* (Shaw 1960). Leivestand et al. (1976) suggest that fish death in acid water is caused by failure of ionic regulation. Alternatively, or in conjunction with ionic imbalance, acid–base imbalance in the blood possibly is the lethal factor. Rainbow trout exposed to pH 4.0 showed decreased pH and CO_2 in the blood (Neville 1979). *Orconectes virilis* in this study elevated the DIC content of low-HCO_3^- media significantly higher than did crayfish in media with normal DIC levels. This may indicate a higher rate of loss of metabolic CO_2 by crayfish in low pH.

In spite of the decrease of the pH of the epilimnion of L223 to as low as 5.5 in 1978, the population of *O. virilis* appeared unchanged in numbers of individuals, age structure, and fecundity from previous years (I. J. Davies unpublished data). Nevertheless, unless

the population possesses acclimatization mechanisms the proposed pH drop in 1979 by an additional 0.3 to 0.5 pH units is expected to stress the population and to affect its size and structure.

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Effects of Acidification on Mobilization of Heavy Metals and Radionuclides from the Sediments of a Freshwater Lake

D. W. SCHINDLER, R. H. HESSLEIN, AND R. WAGEMANN

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

AND W. S. BROECKER

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York, NY 10964, USA

SCHINDLER, D. W., R. H. HESSLEIN, R. WAGEMANN, AND W. S. BROECKER. 1980. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. Can. J. Fish. Aquat. Sci. 37: 373–377.

Large (10 m) diameter enclosures were sealed to the sediments in 2–2.5 m of water in Lake 223. Two tubes were held at control pH (6.7–6.8), one was lowered to pH 5.7 and one to pH 5.1, using H_2SO_4. Aluminum, manganese, zinc, and iron were released from lake sediments at pH 5 and 6. Concentrations of zinc in the overlying water column exceeded 300 $\mu g \cdot L^{-1}$. Radioisotopes of several heavy metals added to the water of the enclosure showed the following: all metals were removed from the water at log-linear rates, with half-times of 5–25 d. Acidification caused several metals to become more soluble, including Fe-59, Co-60, Mn-54, and Zn-65. Solubility of V-48 and Hg-203 decreased with increasing acidity. Acidification also slowed the loss to sediments of Mn-54 and Zn-65. Losses of Ba-133, Se-75, Cs-134, and V-48 were more rapid under acid conditions. The fractions of any isotope retained by a 0.45-μm filter, activated charcoal and mixed-bed ion exchange resin remained constant throughout the experiment at any given pH.

Key words: sediment–water interactions, heavy metals, radionuclides, lake acidification

SCHINDLER, D. W., R. H. HESSLEIN, R. WAGEMANN, AND W. S. BROECKER. 1980. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. Can. J. Fish. Aquat. Sci. 37: 373–377.

Des enceintes de grand diamètre (10 m) furent scellés aux sédiments dans 2 à 2,5 m d'eau dans le lac 223. Deux tubes furent maintenus à un pH témoin (6,7–6,8); l'un fut abaissé au pH 5,7 et l'autre, au pH 5,1, à l'aide de H_2SO_4. Aux pH 5 et 6, l'aluminium, le manganèse, le zinc et le fer sont libérés des sédiments du lac. Les concentrations de zinc dans la colonne d'eau sus-jacente dépassent 300 $\mu g \cdot L^{-1}$. Les radioisotopes de plusieurs métaux lourds ajoutés à l'eau dans l'enceinte montrent la caractéristique suivante : tous les métaux sont enlevés de l'eau à des taux logarithmiquement linéaires, avec demi-vie de 5–25 jours. Par suite de l'acidification, plusieurs métaux deviennent plus solubles, y compris Fe-59, Co-60, Mn-54 et Zn-65. La solubilité de V-48 et H-203 diminue avec l'augmentation de l'acidité. L'acidification ralentit aussi la perte vers les sédiments de Mn-54 et Zn-65. Les pertes de Ba-133, Se-75, Cs-134 et V-48 sont plus rapides dans des conditions acides. Les fractions de tout isotope retenues par filtre de 0,45 μm, charbon activé et résine d'échange ionique à fond mixte, demeurent constantes tout au long de l'expérience à tout pH donné.

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THE acidification of natural waters by industrially contaminated precipitation has now affected large areas of Scandinavia and eastern North America (for example, Likens and Bormann 1974; Beamish and Harvey 1972; Wright and Gjessing 1976). Increased concentrations of heavy metals have also been found in these acidified waters. The increases have usually been attributed either to industrial contamination of precipitation (Kramer 1976; Beamish et al. 1975; Wright and Gjessing 1976) or to leaching from soils in the drainage basins of lakes (Malmer 1976).

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It seemed to us that at low pH, much of the observed increase in heavy metals could be leached from lake sediments, in addition to the above two sources. It would be impossible to distinguish the three sources of metals in areas where acid and heavy metal contamination from industrial emissions are widespread. In addition, we believed that radioactive metals from fallout or from nuclear power plants in noncalcareous areas might have increased mobilities in acidified waters.

We therefore decided to test the extent to which acidification would mobilize heavy metals and radionuclides from lake sediments by conducting experiments in the Experimental Lakes Area, where atmospheric deposition of heavy metals is negligible, and

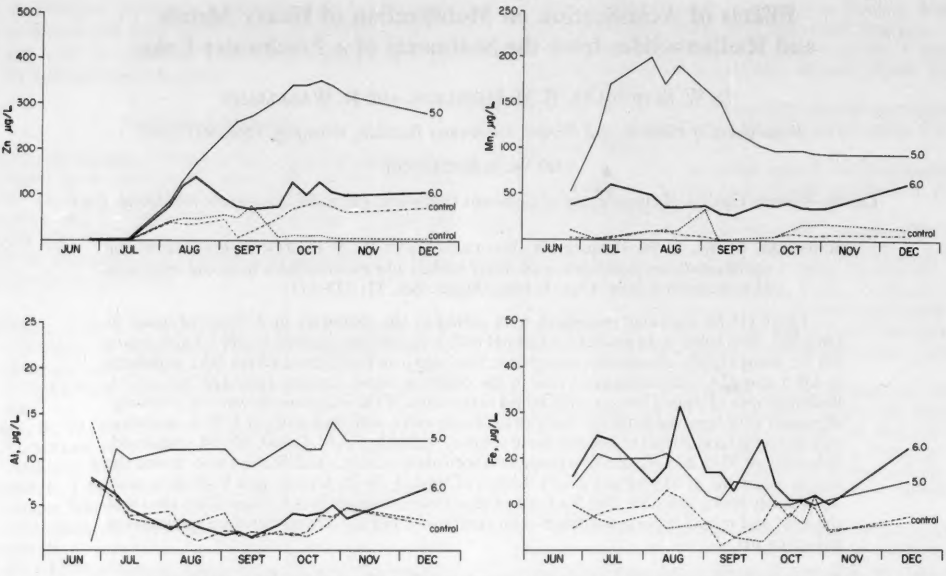

FIG. 1. Concentrations of zinc, manganese, aluminum, and iron observed in four 10 m diameter tubes which were open to the littoral sediments of Lake 223, and which were held at different pH values, as indicated at the right of each panel. Control tubes were at pH 6.7–6.8.

where the mean annual pH of precipitation is 4.9–5.0, a value which has not caused a detectable increase in the acidity of lakes in the area during the past 8 yr.

Materials and Methods

The experiments were conducted in large circular enclosures constructed of cross-laminated polyethylene, 10 m diameter and 2.0–2.5 m deep. The bottoms of the tubes were sealed to the sediments, which were of quartz sand of variable grain size, by piling rocks on the edge of the tube material. Details of construction are given by Schindler et al. (1979). Experiments were done in Lake 223 (Schindler et al. 1980).

During the period May 28 to June 1976, two tubes were acidified to pH 5.1 and 5.7, respectively, using H_2SO_4. Occasional additions of acid were made during the course of the experiment to hold the pH values of the tubes to within 0.2 units of these values. Two adjacent tubes were allowed to remain at normal pH (6.7–6.8).

Samples for heavy metal analyses were taken at weekly intervals, using specially cleaned 1-L linear polyethylene bottles, filtered on precleaned 0.22-µm millipore membranes in a teflon-coated filter apparatus, and acidified to pH 1 using redistilled HNO_3. Samples were analyzed for zinc, cobalt, lead, iron, manganese, cadmium, copper, and aluminum by flameless atomic absorption spectrometry.

On June 17, 1976, a multi-isotope addition was made to each tube. 0.5 mCi (1 Ci = 37 GBq) of each of the following isotopes were added to each tube: zinc-65, cobalt-60, cesium-134, mercury-203, manganese-54, iron-59, thorium-228, chromium-51, barium-133, vanadium-48, selenium-75, and arsenic-74. 0.5 µCi of each metal lead-210, plutonium-238, plutonium-239, americium-241, neptunium-237, and curium-242 were also added to each enclosure. Water in each tube was mixed for several minutes with a small electric outboard motor while isotopes were added to ensure that mixing was complete.

Samples of water for radioassay were taken on days 1, 3, 5, 9, 14, 20, 28, and 41. Each sample was first filtered through a millipore 0.45-µm membrane 47 mm in diameter. Filtrates were then passed first through activated charcoal and then mixed-bed anion and cation exchange resin. Fractions to be analyzed for gamma emitting metals were placed in plastic petri plates with locking lids, then assayed on a Princeton Gamma Tech lithium drifted germanium detector, with a rated efficiency of 13% connected to a Canberra 8100 pulse height analyzer and a Hewlett-Packard computer. Samples were counted for 1–2 h, depending on activity.

Samples requiring chemical separations and analysis by alpha spectrometry were sent to Lamont-Doherty Geological Observatory; results will be reported elsewhere (P. Santschi unpublished data).

pH values were determined with a Radiometer model 29 pH meter and a combination electrode, standardized daily in buffers of several pH values.

Results

Stable Metals

Four stable metals increased at higher acidity: Zn, Mn, Al, and Fe (Fig. 1). Cu, Cd, Co, Cr, and Pb were usually below the limit of detection ($<1\ \mu g \cdot L^{-1}$).

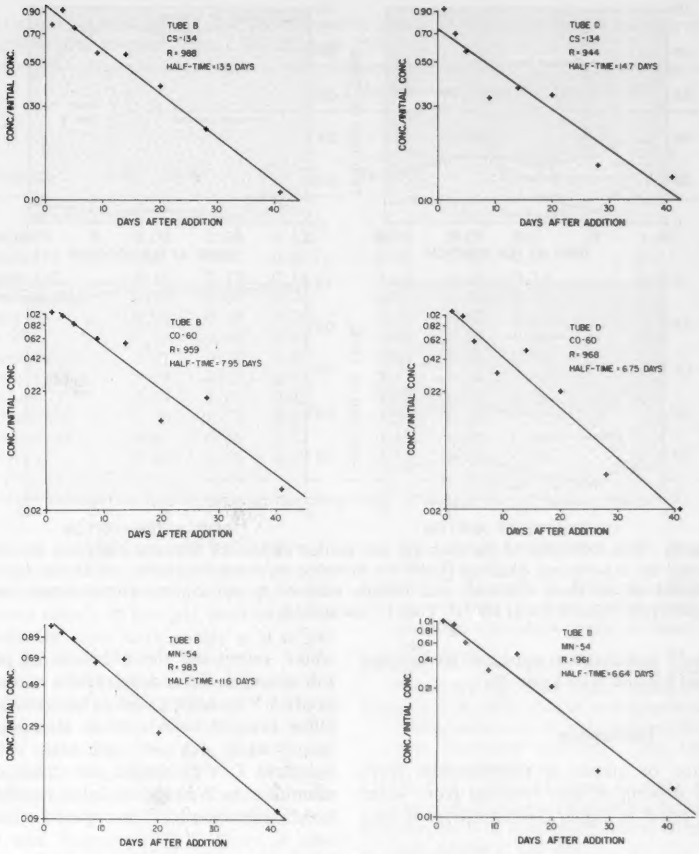

FIG. 2. Selected examples of the log-linear disappearance of isotopes from the tubes B (pH 5.1) and D (pH 6.7). Data represents particulate plus dissolved fractions.

RADIONUCLIDES

All of the radionuclides disappeared from the water at a log-linear rate (Fig. 2). Half times for removal were 5–25 d (Table 1). The approximate significance of changes in the loss rates due to acidification were determined by comparing removal rates from tubes B and C (acid tubes) with those of tubes D and E (control tubes), using Student's *t*-test. Results are given in Table 1.

Acidification slowed the loss of Mn-54 ($P > 0.9$) and probably Zn-65 ($P > 0.7$). It appeared to speed the disappearance from the water column of V-48 and Se-75 ($P > 0.8$), and possibly Ba-133 and Cs-134 ($P > 0.7$). For other isotopes, the probability that acidification affected loss rates was very low (Table 1).

The partition between particulate and dissolved forms varied greatly among isotopes, even at control pH values (Table 2). Fe-59, Co-60, Mn-54, Hg-203, and Zn-65 were 54–99% on particulates at pH 6.7–6.8. In contrast, As-74, Ba-133, and Cs-134 remained largely in dissolved forms. The proportions of other isotopes were intermediate. An anomalous result for Th-228 in tube D made interpretation for that element impossible. In all tubes, the partitioning of isotope between suspended and dissolved phases occurred between addition and the first sampling, then remained constant throughout the period studied (Fig. 3).

The partition between dissolved and particulate phases was significantly affected by acidification in several cases. The proportion on particulates of Co-60, Mn-54, Zn-65, and Ba-133 was less in acid than in control tubes ($P > 0.8$ in all cases). On the other hand, acidification caused an increased proportion of V-48 and Hg-203 to bind to particulates ($P > 0.9$ and >0.7, respectively). The distribution of other isotopes was not significantly altered by acidification, although

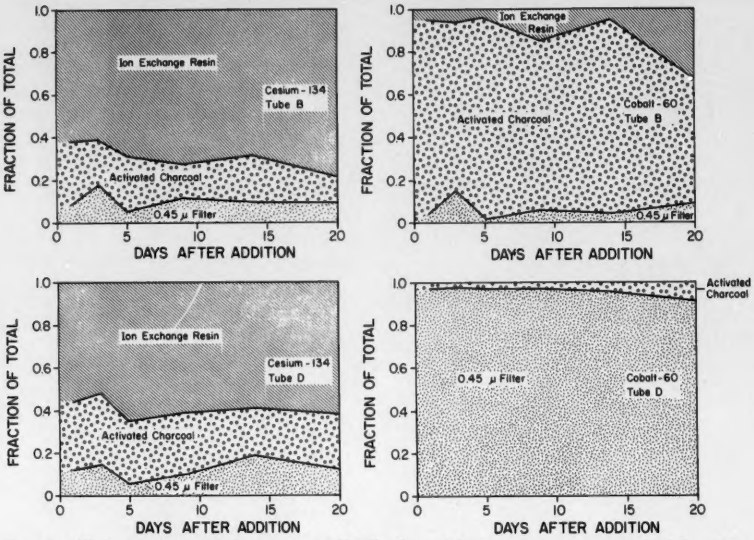

FIG. 3. Two examples of the constant distribution of isotope between analytical fractions during the experiment. Shading (from the bottom) represents particles >0.45 μm, isotope retained on activated charcoal, and isotope retained by mixing-bed ion exchange resin, respectively. Tube B was at pH 5.1. Tube D was at pH 6.7.

in the case of Fe-59 mobilization appeared to increase greatly between pH 5.7 and 5.1 (Table 2).

Discussion

The mobilization of metals or radionuclides from sediments, or the slowing of their removal from water to sediments, will result in higher concentrations in lake water, increasing the exposure of pelagic organisms. For example, the concentrations of stable zinc observed at pH 5.1 are toxic to fish in laboratory studies (Sprague 1964; Grande 1966). While aluminum concentrations in our study did not reach toxic values, Cronan and Schofield (1979) found that toxic concentrations of aluminum in Adirondack lakes resulted from acidification. While the effects on organisms of increased isotope

TABLE 1. Half removal times (in days) for gamma emitting isotopes. 10 m diameter tubes Lake 223, June 1976.

	Tube letter and mean pH, day 0 to 20								
				Control					
Isotope	B(5.12)		C(5.73)	D(6.72)		E(6.82)	acid $\bar{x}$ / control $\bar{x}$	t, df = 2	P[a]
		$\bar{x}$			$\bar{x}$				
Arsenic-74	11.3	12.2	13.0	19.9	16.2	12.4	0.8	−1.04	>0.6
Barium-133	14.6	12.8	10.9	18.5	16.8	15.0	0.8	−1.57	>0.7
Cesium-134	13.5	12.1	10.6	14.7	14.5	14.2	0.9	−1.63	>0.7
Chromium-51	6.4	10.6	14.7	11.2	12.4	13.5	0.8	−0.42	<0.5
Cobalt-60	8.0	7.1	6.2	6.8	6.7	6.6	1.1	0.44	<0.5
Iron-59	8.6	7.2	5.5	10.9	9.0	7.1	0.8	−0.80	<0.5
Manganese-54	11.6	10.6	9.6	6.6	6.7	7.0	1.6	3.73	>0.9
Mercury-203	16.3	13.7	11.1	12.8	12.7	12.6	1.1	0.38	<0.5
Selenium-75	21.7	18.6	15.4	25.0	25.1	25.1	0.8	−2.06	>0.8
Thorium-228	11.1	10.8	10.5	9.6	10.6	11.5	1.0	0.25	<0.5
Vanadium-48	5.4	5.6	5.8	12.7	10.3	7.9	0.6	−1.95	>0.8
Zinc-65	12.7	11.2	9.7	8.5	8.6	8.6	1.3	1.77	>0.7

[a]P = probability that acid had an effect on removal time.

TABLE 2. Mean fraction of gamma activity in seston retained by a 0.45-μm membrane filter. Values are averages for the first 20 d of the experiment in 10 m diameter tubes, Lake 223, June 1976.

		Tube letter and mean pH, day 0 to 20								
					Control					
½ Removal time (days)	Isotope	B(5.12)		C(5.72)	D(6.72)		E(6.82)	acid $\bar{x}$ / control $\bar{x}$	t, df = 2	P[a]
			$\bar{x}$			$\bar{x}$				
14	Arsenic-74	0.02	0.08	0.15	0.10	0.05	0.0	1.6	0.43	<0.5
15	Barium-133	0.0	0.02	0.03	0.10	0.08	0.05	0.2	−2.06	>0.8
13	Cesium-134	0.10	0.12	0.14	0.12	0.14	0.15	0.9	−0.60	<0.5
12	Chromium-51	0.54	0.66	0.79	0.37	0.52	0.67	1.3	0.74	<0.5
7	Cobalt-60	0.06	0.20	0.35	0.97	0.92	0.88	0.2	−4.74	>0.95
8	Iron-59	0.63	0.80	0.98	0.99	0.99	0.99	0.8	−1.06	>0.6
9	Manganese-54	0.01	0.10	0.20	1.00	0.84	0.68	0.1	−3.95	>0.9
13	Mercury-203	0.58	0.62	0.67	0.57	0.54	0.52	1.2	1.55	>0.7
22	Selenium-75	0.37	0.41	0.45	0.39	0.37	0.35	1.1	0.89	<0.5
11	Thorium-228	0.30	0.32	0.35	0.0	0.20	0.41	1.6	0.58	<0.5
8	Vanadium-48	0.61	0.66	0.73	0.48	0.46	0.44	1.4	3.50	>0.9
10	Zinc-65	0.05	0.14	0.23	0.75	0.62	0.50	0.2	−3.15	>0.8

[a] P = probability that acidification had an effect on the proportion of isotope on particulate matter.

concentrations due to acidification are not known, it seems likely that higher concentrations of some isotopes in the food chain would result. In only a few cases has the toxicity of heavy metals at low pH been explored. Many heavy metals are more toxic under acid conditions (for example, Schofield 1976).

Metals and isotopes which have atmospheric or terrestrial sources in addition to being leached from lake sediments deserve special concern. In addition to being leached from lake sediments, Zn from atmospheric emissions is deposited in large quantities for distances of several kilometres around large smelters (Kramer 1976; W. G. Franzin, Freshwater Institute, unpublished data; McFarlane and Franzin 1978). There is also some indication that it may be leached from terrestrial soils (Malmer 1976).

Schofield (1976) has found that Al in large quantities is mobilized from terrestrial soils by extremely acid spring snowmelt. He found that the combination of high acidity and high Al was acutely toxic to fishes, causing rupture of gill filaments.

It is clear from the above that a much more complete picture must be developed of interactions between acid deposition and terrestrial, atmospheric, and aquatic sources of metals and radionuclides before coherent plans can be formulated for controlling the development of smelting and energy producing facilities in the airshed of the Precambrian Shield.

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Fates of Metal Radiotracers Added to a Whole Lake: Sediment–Water Interactions

R. H. HESSLEIN[1] AND W. S. BROECKER

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York, NY 10964, USA

AND D. W. SCHINDLER

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

HESSLEIN, R. H., W. S. BROECKER, AND D. W. SCHINDLER. 1980. Fates of metal radiotracers added to a whole lake: sediment–water interactions. Can. J. Fish. Aquat. Sci. 37: 378–386.

A whole-lake radiotracer experiment with the isotopes Se-75, Hg-203, Cs-134, Fe-59, Co-60 was carried out in Lake 224 of the Experimental Lakes Area, northwestern Ontario. The results of the analyses of isotopes in the water column show an exponential decrease in concentration with time. The rate of loss from the water column does not show dependence on the affinity of the isotope for suspended particulate material. This affinity ranged from 98% for Fe-59 to <1% for Cs-134 over the first 65 d. The major sink for isotopes lost from the water column is the sediments. Ratios of isotopes in the sediments show that those isotopes associated with suspended particulates more easily reach deep sediments than those in dissolved form which are restricted by the thermocline to adsorption to epilimnion sediments.

Key words: Experimental Lakes Area, metal and radioisotope budgets, environmental fate of radionuclides

HESSLEIN, R. H., W. S. BROECKER, AND D. W. SCHINDLER. 1980. Fates of metal radiotracers added to a whole lake: sediment–water interactions. Can. J. Fish. Aquat. Sci. 37: 378–386.

Une expérience avec radiotraceurs, couvrant un lac en entier, a été effectuée dans le lac 224 de la région des lacs expérimentaux, dans le nord-ouest de l'Ontario, utilisant les isotopes Se-75, Hg-203, Cs-134, Fe-59 et Co-60. Les analyses des isotopes dans la colonne d'eau indiquent une diminution exponentielle de concentration avec le temps. Le taux de perte depuis la colonne d'eau ne dépend pas de l'affinité de l'isotope pour la matière particulaire en suspension. Cette affinité varie de 98% pour Fe-59 à <1% pour Cs-134 pendant le premier 65 jours. Les sédiments sont le principal réceptacle des isotopes provenant de la colonne d'eau. Les rapports des isotopes dans les sédiments montrent que les isotopes associés aux particules en suspension atteignent plus facilement les sédiments de profondeur que les isotopes dissous qui, à cause de la thermocline, sont limités à l'adsorption aux sédiments de l'épilimnion.

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A program to study the fates in lakes of radioisotope tracers of heavy metals, interesting both as industrial and nuclear power effluents, has been underway since the summer of 1975. The principal objective of this study is to gain an understanding of the pathways and rates of movement of the radioisotopes in the whole-lake systems. This understanding in conjunction with work on the toxic effects of heavy metal and nuclear effluents should provide a means of estimating the potential effects of these effluents in lakes. Early work in enclosures in lakes has been presented by Kipphut (1978), Hesslein et al. (1978), and Schindler et al. (1980).

On July 29, 1976, the epilimnion of Lake 224 was spiked with six gamma emitting isotopes, Se-75 as sodium selenite, Hg-203 as mercuric chloride, Cs-134 as cesium chloride, Fe-59 as iron chloride, Zn-65 as zinc chloride, and Co-60 as cobalt chloride. The purpose of this experiment was to follow these isotopes through the chemical and biological pathways of the lake and to determine rate constants and accumulation factors for various biological and geochemical compartments. These compartments included water, settling particulates, sediments, zooplankton, periphyton, macrophytes, crayfish, lake trout, white sucker, slimy sculpin, and fathead minnows.

The purpose of this paper is to present the preliminary results of the water, sedimentation trap, and sediment analyses. The results of analyses for isotopes in the biological compartments will appear in a future paper. This separate treatment is possible because

[1]Present address: Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6.

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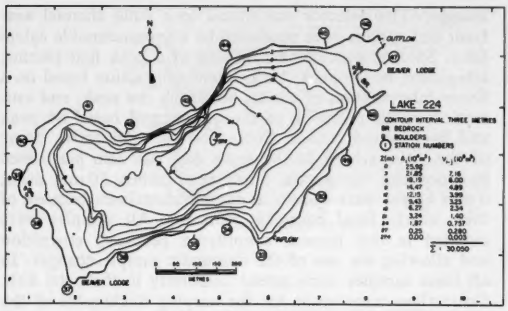

FIG. 1. Lake 224 in the Experimental Lakes Area, northwestern Ontario.

amounts of isotopes in biological compartments other than those sampled by filtering and coring (algae and periphyton) never exceeded 1% of the added amount when integrated over the whole lake.

Methods

STUDY SITE

Lake 224 (Fig. 1) has an area of 26 ha, a maximum depth of 27 m, and a mean depth of 12 m. Its drainage basin in Canadian Shield granite–diorite is overlain with shallow glacial sands and forested primarily by black spruce, jack pine, and birch. Lake sediments to a depth of ~10 m are mostly sandy, often overlain with a thin covering (<5 mm) of loose organic material. Clay underlies 10–20 cm of sand in some areas. There are many large boulders. At depths greater than 10 m the sediments are primarily organic ooze. The lake is oligotrophic with a secchi disk visibility of 7 m and a summer chlorophyll *a* concentration of 1.5 μg/L in the epilimnion, primarily as chrysophytes.

SPIKE PROCEDURE

Two hundred to 500 mCi (1 Ci = 37 GBq) of each isotope as the salts previously listed were mixed into a metal barrel containing 10 L of water. The barrel, mounted on a raft, was towed around the lake by a motor boat while the solution dribbled into the lake from a tube emptying just below the surface. The rate of dribbling was slow, so this procedure required about 45 min to complete. The barrel was then refilled with water and the operation repeated. This method of addition allowed us to distribute the spike throughout the epilimnion of the lake, thus avoiding problems in interpretation which might result from a nonuniform distribution. Earlier mixing experiments (Quay 1977) with tritium as a tracer had shown that we could expect a horizontally uniform distribution within about 24 h of addition. This was borne out by measurements on the first day after the spike. All handling procedures were done directly or supervised by a health physics expert of Atomic Energy Canada Ltd. from the Whiteshell Nuclear Research Establishment. The total effect of the gamma emitting isotopes when diluted to the epilimnion of the lake was ~10% of the maximum permissible concentration (MPC) allowable in drinking water as established by the International Atomic Energy Agency.

WATER SAMPLES

Water samples (2 L per depth) were taken in a 1-m-long Van Dorn bottle at the center of the lake and poured into linear polyethylene bottles. On returning to the laboratory the samples were filtered through a 0.45-μm membrane filter (cellulose acetate). These filters had been tested for adsorption of dissolved isotopes and this was found to be insignificant. Glass fiber filters were unsuitable as they adsorbed significant quantities of Cs-134. Because of this affinity of Cs-134 for porous glass, stainless steel filter supports were used rather than fritted glass. The membrane filters were prewashed by filtering 100 mL of distilled water to remove detergent surfactants (H. J. Simpson, Lamont Doherty Geol. Obs., personal communication).

The filtered water was then passed in sequence through columns (9 mm diam × 40 mm) of activated charcoal (Fisher Activated Coconut Charcoal 50–200 mesh) and mixed cation and anion exchange resins (Dowex 50w-x12 100–200 mesh, 1-x8 100–200 mesh). The activated charcoal is an effective adsorber of nonpolar species. Tests showed that a small percentage of some of the isotopes occasionally passed through these extractions. As a result the remaining sample was cooked at 90°C with 3.5 g/L of potassium bisulphate and 1 cm^3/L of concentrated sulphuric acid for 4 h. A combined ferracyanate–sulphide–hydroxide precipitation was then performed to scavenge any remaining isotope (P. Santchi, Lamont Doherty Geol. Obs., personal communication). The purpose of this procedure was to separate particulate and nonpolar and polar dissolved materials and to reduce the sample volume for increased counting efficiency.

SEDIMENTATION TRAPS

Sedimentation traps (Fig. 2) were constructed from polyethylene bags of 22 cm diameter and 50 cm length. A floatation ring of 0.5 cm OD polyethylene tubing was attached to the open end and the closed bottom was attached by a length of rope to a weight. A loose noose of fishing line was attached at the middle of the bag and flag and cork floated the free end of the noose at the surface as a marker. The trap was retrieved by pulling on the noose, thus closing off the bag and pulling it to the surface. The water and settled particulates were filtered to remove excess water. Most traps were set with the openings just below the epilimnion at various distances from the shore.

SEDIMENT CORES

Sediment cores were taken by two methods: divers took short (20 cm) cores in acrylic tubes (5 cm diam) in the firmer sandier sediments where sufficient light was available (0–10 m depth). Deepwater cores (10–25 m) were taken by a gravity corer (K–B corer) slowly lowered into the very soft profundal sediments. On returning to the lab, cores were sectioned into 0.5–2 cm slices depending on the character of the material. The material was then dried to 80°C and packaged for counting.

ISOTOPE ANALYSES

The activities of gamma emitting isotopes in a sample were determined by gamma spectroscopy performed on a lithium drifted germanium (GeLi) crystal detector. The detector was supplied with samples by an automatic sample

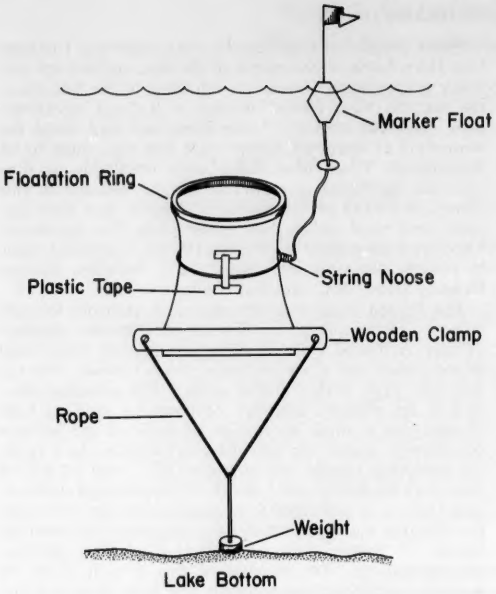

FIG. 2. Sedimentation trap of the type used to catch settling material in Lake 224.

changer. The detector was mated to a 4000 channel analyzer and spectra were processed by a programmable calculator. Spectral processing consisted of a peak find routine, integration of the peak, background subtraction based on a linear interpolation of counts bordering the peak, and estimation of the precision of the net integral based on peak and background counts. Correction for radioactive decay of the isotope back to the spike day was also performed in processing the spectra. Small petri dishes, 50 mm diam, 9 mm height were chosen as sample containers because of their nearly ideal counting geometry. All samples were counted in this format, simplifying geometry correction and allowing the use of the automatic sample changer. In all cases samples were spread uniformly in the petri dish. Corrections were made for the varying thicknesses of the samples, that of a filter to 9 mm. This correction was made by integrating an empirical curve established by measuring a filter standard at various distances from the detector. Self adsorption of gamma rays by the sample proved to be insignificant due to the low density and thinness of the samples. The overall counting efficiency of the system ranged from ~5% at 265 KeV to ~1% at 1333 KeV.

Results

The concentrations of all gamma emitters in unfiltered epilimnion samples showed an exponential decrease with time over the first 65 d. Whole water sample data represent the sum of the fractions: particulate, charcoal extracted, ion exchange extracted, and precipitate. Shown with these data (Fig. 3) are lines represent-

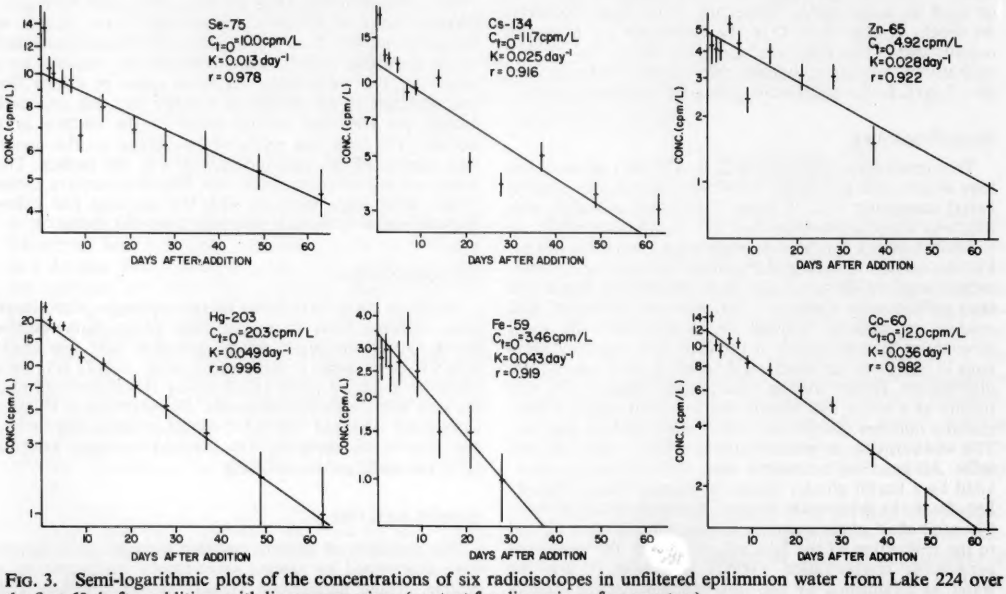

FIG. 3. Semi-logarithmic plots of the concentrations of six radioisotopes in unfiltered epilimnion water from Lake 224 over the first 63 d after addition, with linear regressions (see text for discussion of parameters).

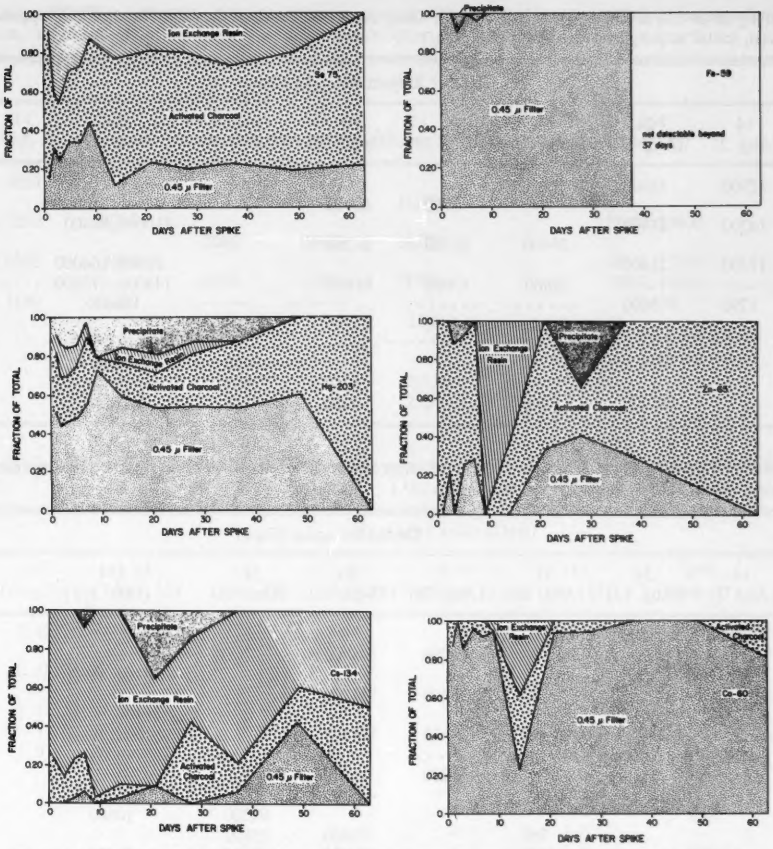

FIG. 4. The distributions of six radioisotopes among separated fractions of epilimnion water from Lake 224.

ing the linear regressions of the log of the concentration versus time. The lines represent an equation of the form: $C = C_{t=0}e^{-kt}$ where C is concentration, t is time, and k is the rate constant for the change in concentration with time. Data from the first day after addition was not included in the linear regressions because samples indicated that some inhomogeneity remained in the epilimnion. This inhomogeneity was not present after the second day after addition.

The rate of loss of the isotopes from the epilimnion can be expressed as a half-time, that is, the time required for the concentration to decrease by a factor of two. This is just $\ln 2/K$ or $0.693/k$. The half-times for loss from the epilimnion are: Se-75, 52.0 d; Hg-203, 14.3 d; Cs-134, 28.1 d; Fe-59, 16.1 d; Zn-65, 24.7 d; and Co-60, 19.5 d.

The distribution of the isotopes between the fractions: particulate, charcoal extracted, ion exchange extracted, and precipitated (Fig. 4), remained fairly constant throughout the first 63 d. The standard deviation (with elimination of the single most aberrant value in some cases) for the fraction or particulates of all isotopes is less than 0.10 except Zn-65 which is 0.16. The loss of activity in certain fractions toward the end of the first 63-d period is probably due to low absolute concentration left in the water causing the concentration in the fraction to be below detection limits in the allotted counting time.

The areal concentrations of isotopes in cores are shown in Tables 1–4. These values are the integrated values over the length of the core (about 5–8 cm); however, in most cases the isotopes were confined to the top 1–3 cm. In many cases there is good agreement between cores at the same or similar depth, but often there are large differences, probably due to the heterogeneity of the surface sediments. Much of this spatial

TABLE 1. Activity of Cs-134 in Lake 224 cores in cpm/m² integrated over length of the core. (Dashed line represents the bottom of the epilimnion, initial activity in water was 59000 cpm/m².)

	Days after spike (Date)							
Depth (m)	14 (Aug. 3)	24 (Aug. 13)	31 (Aug. 20)	37 (Aug. 26)	63 (Sept. 21)	147 (Dec. 14)	254 (Mar. 31)	331 (June 16)
1	17900	33000						
2				35700	62900			
3	14700	27400					110000, 98400	238000, 148000
4			26400	56700	61700			
5	17200	21400					89800, 164000	117000, 94700
6	------	------	22800	37600	91600		145000, 175000	------
7	1790	3000	------	------			166000	88700, 83400
8			4600	6060	24100			
10			1330		------			54000, 45100
15–16						43400	8450	
20–21					4760	17600		
25			890		2900	4331	25200	

TABLE 2. Activity of Co-60 in Lake 224 cores in cpm/m² integrated over length of the core. (Dashed line represents the bottom of the epilimnion, initial activity in water was 60200 cmp/m².)

	Days after spike (Date)							
Depth (m)	14 (Aug. 3)	24 (Aug. 13)	31 (Aug. 20)	37 (Aug. 26)	63 (Sept. 21)	147 (Dec. 14)	254 (Mar. 31)	331 (June 16)
1	7540	13100						
2				13700	25800			
3	8600	11500					54800, 46900	89900, 58500
4			13800	26400	24700			
5	5910	8990					45100, 90100	48400, 39600
6	------	------	9390	18400	44400		81000, 99500	------
7	7000	11000	------	------			99900	40500, 39600
8			12700	31200	26600			
10			14300		------			103000, 81700
15–16						62200	10600	
20–21			760		19400	28800		
25			4210		10600	13100	35400	

TABLE 3. Activity of Zn-65 in Lake 224 cores in cpm/m² integrated over length of the core. (Dashed line represents the bottom of the epilimnion, initial activity in the water was 25100 cpm/m².)

	Days after spike (Date)							
Depth (m)	14 (Aug. 3)	24 (Aug. 13)	31 (Aug. 20)	37 (Aug. 26)	63 (Sept. 21)	147 (Dec. 14)	254 (Mar. 31)	331 (June 16)
1	6060	12300						
2				10800	16400			
3	4040	8060					30400, 23400	59500, 32500
4			8460	16200	14200			
5	5360	6930					20500, 44000	31161, 24700
6	------	------	6810	10100	26800		41500, 33500	------
7	2370	3190	------	------			42200	21500, 23100
8			2910	6140	7280			
10			2990		------			28500, 21700
15–16						16800	1980	
20–21			—		3160	3730		
25			770		3030	—	9910	

TABLE 4. Activity of Se-75 in Lake 224 cores in cpm/m² integrated over length of the core. (Dashed line represents the bottom of the epilimnion, initial activity in the water was 50300 cpm/m².)

	Days after spike (Date)							
Depth (m)	14 (Aug. 3)	24 (Aug. 13)	31 (Aug. 20)	37 (Aug. 26)	63 (Sept. 21)	147 (Dec. 14)	254 (Mar. 31)	331 (June 16)
1	4660	6790						
2				8430	13300			
3	3290	4860					22300, 20600	44600, 27400
4			7060	19600	10200			
5	4040	4000					30000, 17330	28900, 20300
6	------	------	4290	6390	15700		32000, 36400	------
7	3140	2190	------	------			36400	18800, 15200
8			1370	2570	2750			
10			1030		------			25500, 10200
15–16						3910	2480	
20–21			—		2693	3330		
25			—		2350	2370	11000	

TABLE 5. Co-60/Cs-134 ratio in Lake 224 cores. (Dashed line represents the bottom of the epilimnion, Co-60/Cs-134 ratio in spike was 1.02.)

	Days after spike (Date)							
Depth (m)	14 (Aug. 3)	24 (Aug. 13)	31 (Aug. 20)	37 (Aug. 26)	63 (Sept. 21)	147 (Dec. 14)	254 (Mar. 31)	331 (June 16)
1	0.42	0.40						
2				0.38	0.41			
3	0.58	0.42					0.50, 0.48	0.38, 0.40
4			0.52	0.47	0.40			
5	0.34	0.42					0.50, 0.55	0.41, 0.42
6	------	------	0.41	0.49	0.48		0.56, 0.57	------
7	3.9	3.7	------	------			0.60	0.46, 0.48
8			2.8	5.1	1.1			
10			10.7		------			1.90, 1.81
15–16						1.4	1.3	
20–21					4.1	1.6		
25			4.8		3.6	3.0	1.4	

variance can be removed if the data are presented as ratios of concentrations (Tables 5–7) rather than absolute amounts. Cs-134 was chosen as the denominator in these ratios as it showed almost no affinity for the particulate phase (mean of 0.03 on filters over 63 d). Only Se-75, Zn-65, Co-60, and Cs 134 were detectable in cores after 65 d, therefore only these data are presented.

Sediment trap data (Table 8) also show some highly variant values in terms of absolute concentrations. As in the case of the cores much of this variance is removed when the data are expressed as ratios. The higher variance in absolute values is likely due to variance in trapping efficiency and problems in retrieval of the traps.

Discussion

The change in epilimnion whole water concentration of all isotopes with time shows a highly significant correlation when fitted by the first order equation $C = C_{t=0}e^{-kt}$. This suggests that the rates for removing the isotopes from solution are dependent on their concentrations. Two simple processes in the lake which would remove isotopes in this fashion are direct adsorption to sediments and settling of particulates. If these processes operated at different rates one would expect to see a difference in the removal rate of isotopes with high or low affinity for particulate material. No clear relationship is apparent between the rate constant for removal of an isotope and the average fraction of that isotope associated with particulate materials (Fig. 5), although those with a high fraction on particulate material seem to have slightly faster rates of removal. Either the rates of the two processes are similar or a more complicated process is involved.

The data in Tables 1 to 4 strongly support the theory

TABLE 6. Zn-65/Cs-134 ratio in Lake 224 cores. (Dashed line represents the bottom of the epilimnion, Zn-65/Cs-134 ratio in spike was 0.43.)

Depth (m)	Days after spike (Date)							
	14 (Aug. 3)	24 (Aug. 13)	31 (Aug. 31)	37 (Aug. 26)	63 (Sept. 21)	147 (Dec. 14)	254 (Mar. 31)	331 (June 16)
1	0.34	0.37						
2				0.30	0.26			
3	0.27	0.29					0.28, 0.24	0.25, 0.22
4			0.32	0.29	0.23			
5	0.31	0.32					0.23, 0.27	0.27, 0.26
6	------	------	0.30	0.27	0.29		0.29, 0.19	------
7	1.3	1.1	------	------			0.25	0.24, 0.28
8			0.63	1.0	0.30			
10			2.3		------			0.53, 0.48
15–16						0.39	0.23	
20–21					0.66	0.21		
25			0.90		1.0		0.39	

that the two processes of adsorption and settling are responsible for the removal of the isotopes from the water and show that a nonhomogeneous distribution of the isotopes is thereby produced in the sediments. Isotopes showing a high fraction associated with particulate, Fe-59 (0.98) and Co-60 (0.87) are not free to be readily removed by direct adsorption to sediments, but can easily settle with their associated particles. Cs-134 (0.03 on particulate) on the other hand has virtually no association with particles to allow settling, but has a large fraction in solution potentially available for adsorption. The ratios of Co-60/Cs-134 in the cores illustrate this separation due to different pathways (Table 5). Although both isotopes have similar rates of removal from the water (0.036 d^{-1} and 0.025 d^{-1}), Cs-134 is 2.5 times more abundant than Co in the epilimnion sediments and Co-60 is 3–10 times more abundant in sediments below the epilimnion. The inability of Cs-134 to reach deep sediments is clearly shown in Table 1. Cs-134 accumulation in epilimnion sediments is 5–10 times more rapid than in sediments below the thermocline while Co-60 is accumulated at nearly equal rates above and just below the thermocline and at about half that rate in deepwater sediments. Zn-65 which is also removed from the water at a similar rate (0.028 d^{-1}), but has a lower association with particulate (0.21), shows a similar but less dramatic separation relative to Cs-134. Cs-134 is 1.2 times more abundant than Zn-65 in the epilimnion sediments relative to its concentrations in the water while Zn-65 is 1.6–6 times more abundant in deep water sediments. Se-75 shows the same general pattern but is more difficult to compare bcause of its much slower removal rate. Table 1 also shows clearly that, as the thermocline is eroded in the fall and Cs-134 remaining in the water comes into contact with deeper water sediments, the abundance of Cs-134 in those previously isolated sediments rises rapidly.

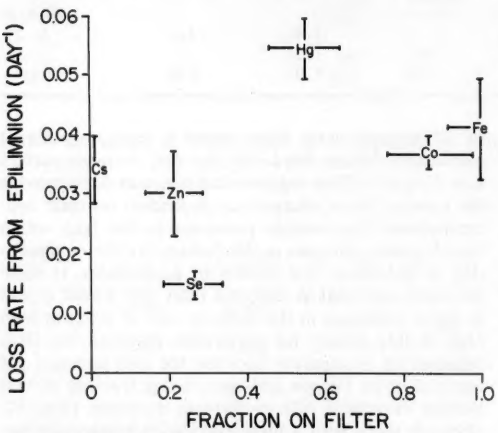

FIG. 5. Rate constant (K) for loss of radioisotope from the epilimnion of Lake 224 plotted against the mean fraction removed by filtration through a 0.45-μm filter over the first 37 d after addition. Error bars indicate 66% confidence interval of mean of fraction on filter for 12 samples.

In spite of the variability of activity accumulated in sediment traps (Table 8) the ratios of isotopes clearly show the greater abundance of isotopes with high affinities for particulate material such as Co-60 as compared to Cs-134. Ratios of isotopes in sediment traps hung at deeper depths are similar for ratios found in deepwater sediments (Tables 5–7). Ratios in traps hung at the bottom of the epilimnion show higher abundance of all isotopes relative to Cs-134 than epilimnion sediments but not as high as deepwater sediments and deeper sedimentation traps. Although there is no clear explanation for this discrepancy it is possible that Cs-134 in shallower traps had adsorbed to material collected from the water. This may also explain the increase in the relative abundance of Cs-134 in the sedimentation

TABLE 7. Se-75/Cs-134 ratio in Lake 224 cores. (Dashed line represents the bottom of the epilimnion, Se-75/Cs-134 ratio in spike was 0.85.)

Depth (m)	Days after spike (Days)							
	14 (Aug. 3)	24 (Aug. 13)	31 (Aug. 20)	37 (Aug. 26)	63 (Sept. 21)	147 (Dec. 14)	254 (Mar. 31)	331 (June 16)
1	0.26	0.21						
2				0.24	0.21			
3	0.22	0.18					0.20, 0.21	0.19, 0.19
4			0.27	0.35	0.17			
5	0.23	0.19					0.19, 0.18	0.25, 0.21
6	------	-----	0.19	0.17	0.17		0.22, 0.21	------
7			------	------			0.22	0.21, 0.18
8			0.30	0.42	0.11			
10			0.77		------			0.47, 0.23
15–16						0.09	0.29	
20–21					0.62	0.18		
25					0.81	0.55	0.44	

traps with time throughout the experiment.

It is somewhat difficult to explain why Cs-134 should appear at all in the sedimentation traps, at least at deeper depths and in the deepwater sediments as it has almost no association with particulate material. It is also surprising that Cs-134, Co-60, and Zn-65 all reach the sediments at a water depth of 25 m in 31 d or less. This requires a settling velocity of at least 0.7 m/d. If one calculates the settling rate of Co-60 using its removal rate and fraction on particulates one arrives at a figure of about 0.18 m/d. One process which would accommodate both of these findings would be a significant transport of metals by fecal pellet settling. Fecal pellets would fall rapidly enough to move isotopes at a rate of 0.7 m/d or greater. In addition, because of their rapid sinking, the concentration of fecal pellets in the epilimnion would be very low thus evading detection on filters. Cs-134 would therefore appear in the sedimentation traps and deepwater sediments but not on the filters. The higher abundance of Co-60 in the epilimnion sediments relative to deepwater sediments could be due to a more or less even distribution of fecal pellet settling with an added increment of fine particles which settle more slowly to the epilimnion sediments. Fecal pellets are of major importance to sedimentation in many natural water bodies (Honjo and Roman 1978; Ferrante and Parker 1977; L. Small, U. of Michigan, personal communication) especially those of considerable depth.

There is no evidence of major redistribution of isotopes through resuspension and settling as has been suggested by others (Davis 1968; Emerson and Hesslein 1973). The concentrations of Se-75, Zn-65, and Cs-134 in deepwater sediments remain low relative to epilimnion sediments through fall overturn (about day 80–110) and winter ice cover. If more than 10% of the epilimnion sediments with adsorbed isotopes had been resuspended and settled uniformly the deepwater sediment concentrations would have risen significantly. There is also no evidence for major "focusing" through sedimentation. Concentrations at 25-m depth are not consistently higher than those at 15- and 20-m depth.

Mass balance calculations 350 d after addition of the isotopes show 54% Se-75, 22% Cs-134, 19% Zn-65, and 23% Co-60 of the added amounts remaining in the water column. Total sediment burden based on sums weighted by area gives 50% Se-75, 112% Cs-134, 82% Zn-65, and 90% Co-60 of the added amounts. This yields sums of 104, 134, 103, and 113% of added amounts present 350 d after addition, which considering errors in integration of concentrations in sediments, is an acceptable balance. Because of the dryness of the 1976–77 season and the small fraction left in the water, outflow losses were not significant.

TABLE 8. Activities of isotopes in sedimentation traps in cpm/m².

Days after spike	Depth (m)	Se-75	Hg-203	Cs-134	Fe-59	Zn-65	Co-60
0–14	7	227	836	410	454	300	737
0–14	7	1370	4840	2290	2090	1640	4380
0–14	7	944	4170	1950	1990	1680	4230
0–14	7	980	3360	1590	1110	1370	4810
14–28	5	282	1500	892	691	654	1530
14–28	5.5	256	1550	867	679	653	1670
14–28	5.5	476	2130	1490	1720	1190	3950
14–28	10	315	1250	333	1140	560	2200
14–28	12	59	334	84	231	103	460
28–42	8	448	1580	1610	1210	1160	3880
28–42	8	405	1560	2150	990	1200	3460
28–42	8	308	1250	1870	680	931	2060
28–42	8	541	2430	2000	738	1250	2320
28–42	10	368	1120	1080	849	925	2840
42–63	9	695	1670	3630	584	1520	3230
42–63	9	409	1220	2390	402	969	2400
42–63	10	313	1110	1300	602	933	2590

Conclusion

The removal rates of the added isotopes Se-75, Hg-203, Cs-134, Fe-59, Zn-65, and Co-60 from the water column of Lake 224 were quite rapid and fairly similar

ranging from 1.3 to 4.9%/d. Both settling of particulate material and direct adsorption to sediments are important mechanisms for this removal. The relative importance of the two pathways depends on the isotopes' affinity for suspended particulate material. Direct adsorption of isotopes to sediments limited the deposition of Cs-134, with no affinity for suspended particulates, primarily to epilimnion sediments; however, we believe that rapidly settling particles, perhaps fecal pellets, transported some Cs-134 to deep water sediments.

The distribution of Co-60 with a high affinity for suspended particulate material showed much less preferential deposition in the epilimnion. This distribution is probably a result of settling of both high and low velocity particles. Other isotopes were intermediate in distribution.

A relatively high percentage of isotopes (50% Se-75, and ~20% Cs-134, Zn-65, and Co-60) remained in the water column one year after addition.

Acknowledgments

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Experimental Study of Trace Metal Chemistry in Soft-Water Lakes at Different pH Levels

TOGWELL A. JACKSON

Department of the Environment, Inland Waters Directorate, Freshwater Institute, Winnipeg, Man. R3T 2N6

GEORGE KIPPHUT[1]

Lamont-Doherty Geological Observatory of Columbia University, Palisades, NY 10964, USA

RAYMOND H. HESSLEIN AND DAVID W. SCHINDLER

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

JACKSON, T. A., G. KIPPHUT, R. HESSLEIN, AND D. W. SCHINDLER. 1980. Experimental study of trace metal chemistry in soft-water lakes at different pH levels. Can. J. Fish. Aquat. Sci. 37: 387–402.

The biogeochemistry of Hg, Zn, Co, Fe, Mn, Cr, V, Th, Ba, Cs, As, and Se in two soft-water lakes of the Canadian Shield was investigated by means of carrier-free γ-emitting isotopes introduced into limnocorrals in which the pH of the water was varied from 6.8 to 5.1. The residence times of the radionuclides in the water were determined, and the partitioning of the nuclides among different metal-binding agents in the water and sediments was studied with the aid of membrane filtration, dialysis, solvent extractions, and fractionation on Sephadex columns.

Metal behaviour varied systematically with metal properties. Metals of high crystal field stabilization energy, high electronegativity, or small ionic radius were most readily scavenged by >0.45 μm suspended particles and dispersed colloids in the water, disappeared most rapidly from the water column, and were preferentially accumulated by sedimentary binding agents, including organic substances. Which property of a metal had the dominant effect on metal behavior depended on environmental factors, such as the ambient pH and the nature of the binding agents. Thus, Hg was removed fairly rapidly from the water at pH 6.7–6.8 owing to its high electronegativity but was removed more slowly than any other metal at pH 5.1 owing to its large ionic radius.

Most metals were strongly chemisorbed or complexed by mud and organic ooze, and only Ba could be redissolved by cation exchange. Therefore, 0.1 N NaOH and 0.1 N or 1 N HCl were more efficient extractants than H_2O, 1 N $CaCl_2$, or benzene/methanol, and the metals were largely nonextractable by any of these solvents. The major NaOH-extractable metal-binding agents were the higher molecular weight fractions of a group of organic (probably humic) substances characterized by a UV absorption band at $\simeq$265 nm. The affinity of metals for the highest molecular weight "humic" (NaOH-extractable) substances increased with electronegativity, being greatest for Hg. Hg and Se were nonextractable by HCl but were the elements most readily extracted by NaOH, implying exceptionally strong covalent bonding by humic matter. The more electropositive metals were more readily extracted by HCl than by NaOH, suggesting weaker, less highly covalent bonding or preferential sorption by acid-soluble hydrated oxides.

Acidification of lake water to pH 5.1 interfered with accumulation of Hg and other metals by organic ooze, probably owing in part to interference with the deposition or formation of the complexing agents with the 265 nm absorption band. Acidification also lowered the concentration of NaOH-extractable colloidal phosphate in the ooze but had no effect on NaOH-extractable orthophosphate content.

Key words: metals, lakes, chemistry, pH, humic matter, organic matter, phosphorus, radioactive tracers, acidification

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La biogéochimie de Hg, Zn, Co, Fe, Mn, Cr, V, Th, Ba, Cs, As et Se dans deux lacs d'eau douce du Bouclier canadien a été étudiée au moyen d'isotopes sans porteur émettant des rayons

[1]Present address: Marine Sciences Program, University of North Carolina, Chapel Hill, NC 27514, USA.

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γ introduits dans des enceintes limnétiques dans lesquelles on faisait varier le pH de l'eau entre 6,8 et 5,1. Nous avons mesuré le temps de résidence des radionuclides dans l'eau et étudié, par filtrage sur membrane, dialyse, extractions par solvants et fractionnement sur colonne Sephadex, le fractionnement des nuclides parmi différents agents fixateurs de métaux dans l'eau et les sédiments.

Le comportement des métaux varie systématiquement selon leurs propriétés. Les métaux à haute énergie de stabilisation d'un champ de cristal, forte électronégativité, ou petit rayon ionique sont les plus facilement fixés par les particules en suspension et colloïdes dispersés de plus de 0,45 μm dans l'eau, disparaissent le plus rapidement de la colonne d'eau et sont accumulés de préférence aux autres par les agents fixateurs du sédiment, y compris les substances organiques. Les facteurs de l'environnement, tels que pH ambiant et nature des agents fixateurs, déterminent quelle propriété d'un métal a l'effet dominant sur le comportement de ce dernier. C'est ainsi que Hg est enlevé assez rapidement de l'eau à un pH de 6,7–6,8 par suite de sa forte électronégativité, mais est enlevé plus lentement que tout autre métal au pH 5,1 à cause de son grand rayon ionique.

La plupart des métaux sont fortement chemisorbés ou complexés par vase et boue organique, et seul Ba peut être redissout par échange de cations. Pour cette raison, NaOH 0,1 N et HCl 0,1 N ou 1 N sont des extracteurs plus efficaces que H_2O, $CaCl_2$ 1 N, ou le benzène/méthanol, et les métaux, pour la plupart, ne peuvent être extraits par aucun de ces solvants. Les principaux agents fixateurs des métaux extractibles par NaOH sont les fractions à poids moléculaire élevé d'un groupe de substances organiques (probablement humiques) caractérisées par une bande d'absorption des rayons UV à $\simeq$265 nm. L'affinité des métaux pour les substances «humiques» (extractibles au NaOH) de poids moléculaire le plus élevé augmente avec l'électronégativité, atteignant un maximum avec Hg. Hg et Se ne sont pas extractibles par HCl, mais sont les éléments les plus facilement extraits par NaOH, ce qui laisse supposer une liaison covalente exceptionnellement forte avec la matière humique. Les métaux les plus électropositifs sont plus facilement extraits par HCl que par NaOH, ce qui suggère une liaison moins fortement covalente ou une sorption préférentielle par les oxydes hydratés solubles aux acides.

L'acidification de l'eau de lac à un pH de 5,1 nuit à l'accumulation de Hg et autres métaux par la boue organique, probablement dû en partie à une interférence avec déposition ou formation d'agents complexants à bande d'absorption de 265 nm. L'acidification réduit également la concentration de phosphate colloïdal extractible par NaOH dans la boue, mais est sans effet sur le contenu en orthophosphate extractible par NaOH.

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THE biological effects of trace elements in an aquatic environment depend on the forms as well as the concentrations of the elements. Thus, the binding of trace-metal ions by organic or inorganic complexing agents and sorption sites may (1) decrease heavy-metal toxicity by sequestering the metals in solution (Fogg and Westlake 1955; Steemann-Nielsen and Kamp-Nielsen 1970; Steemann-Nielsen and Wium-Andersen 1970; Zitko et al. 1973; Pagenkopf et al. 1974; Milanovich et al. 1975) or by precipitating them (Sprague 1964; Jernelöv and Lann 1973), (2) increase heavy-metal toxicity by creating particularly dangerous, bio-available species like monomethylmercury (Jernelöv and Lann 1973), (3) enhance the availability of micronutrients by stabilizing them in solution (Johnston 1964; Barber and Ryther 1969; Schnitzer 1969) or (4) cause fixation of micronutrients in relatively unavailable forms (Goodman and Cheshire 1976).

Inasmuch as complex formation and sorption are influenced by environmental conditions such as pH, the effects of "acid rain" on the biogeochemistry of trace metals in poorly buffered inland waters deserve careful scrutiny. Acidification of lakes by airborne H_2SO_4 and other strong acids emanating from industrial sources is a serious, widespread problem. Although the effect of acidification on trace-element biogeochemistry is not well known, this phenomenon is thought to aggravate the harmful ecological impact of mercury contamination by promoting the production of monomethylmercury (Jernelöv and Lann 1973; Brouzes et al. 1977). Acidification may also increase the toxicity of copper and zinc to freshwater fish (Sprague 1964; Pagenkopf et al. 1974).

This report presents the results of a comparative study of the biogeochemical behavior of a wide variety of trace metals (true metals and metalloids) in soft-water lakes at both natural and artificially lowered pH, with emphasis on the forms of the metals and the relative affinities of the metals for different binding agents in the water and sediments. We sought to determine whether the partitioning of trace metals in such environments varies in a systematic, predictable fashion with respect to the properties of these metals, the nature of the binding agents, and the pH of the water. Such an approach could provide a basis for predicting and controlling the fate of trace elements in soft-water lakes and for relating empirical observations to the theories of coordination chemistry.

γ-emitting isotopes of the metals were introduced into both pristine and acidified lake waters confined by limnocorrals, and the partitioning of these isotopes among different operationally defined components of

the water and sediments was investigated. The advantages of γ-emitting tracer analysis are (1) the absence of contamination from natural nuclides and (2) the high sensitivity of the method, allowing investigation at very low concentrations.

The investigation was part of a larger study treated elsewhere by Schindler et al. (1980a, b), Hesslein et al. (1980), and G. W. Kipphut and R. H. Hesslein (unpublished data). G. W. Kipphut and R. H. Hesslein (unpublished data) and Schindler et al. (1980a) demonstrated that the radionuclides introduced into the limnocorrals were scavenged by seston and bottom sediments, and tended to disappear from the water column. But the efficiency of uptake by seston and the rate of removal from the water column varied considerably, being generally greatest for certain transition metals and least for highly electropositive metals like Ba and Cs and for metalloids like Se and As under both "normal" and acidified conditions. All metals were removed from the water at exponential rates, but the time required for removal of half the quantity of nuclide added ranged from less than a week to nearly a month. Acidification of the water to pH 5.1 inhibited the uptake of most trace metals by seston but had no effect on uptake of Hg. Nevertheless, acidification retarded the removal of Hg from the water column, with the remarkable result that at pH 5.1 Hg had a longer residence time in the water than any other true metal; in this regard Hg, surprisingly, behaved like the highly electropositive metals Ba and Cs (Schindler et al. 1980a).

In the report presented here, an attempt is made to extend and systematize these findings and to understand the chemical mechanisms involved.

Materials and Methods

Field Work

The experiments were conducted in two small soft-water lakes (Lakes 303 and 223) in the Experimental Lakes Area of northwestern Ontario, a region covered by boreal forest and underlain by granitic rocks of the Canadian Shield (see special issue of *J. Fish. Res. Board Can.*, 28(2), 1971).

In August, 1975, five limnocorrals consisting of plastic tubes ≃1 m in diameter were set up in Lake 303 at a water depth of 1.1 m. The chemical environment was varied in these tubes (G. W. Kipphut and R. H. Hesslein unpublished data), but only the "control" tube, whose environment was not manipulated, is treated in this report. On August 20 a 50-mL aliquot of a 0.01 N HCl solution containing the following γ-emitting metal isotopes in "carrier-free" form was injected just below the surface of the water:

Isotope	Total initial activity (Bq × 10^{-6})*
^{203}Hg	4.44
^{65}Zn	0.74
^{60}Co	0.74
^{54}Mn	5.18
^{59}Fe	7.77
^{51}Cr	31.8
^{134}Cs	0.74
^{74}As	[too low for accurate measurement].

*Bq = becquerels (disintegrations per second). One curie = 3.7 × 10^{10} Bq.

For the analytical work reported here, a water sample was collected just beneath the surface 44 h after addition of the isotopes, and a grab sample of bottom mud was collected on the 14th day.

In May, 1976, limnocorrals 10 m in diameter were placed in Lake 223 at a depth of 2–2.5 m. In one of the limnocorrals (the "acidified tube") the water was artificially acidified to pH ≃ 5.1 by addition of H_2SO_4, and in three other corrals (the "control tubes") the pH was left at its natural level (≃6.7–6.8). Six white suckers (*Catostomus commersoni*) were introduced into each limnocorral except one (the "control tube without fish"). Sediment traps consisting of plastic bags suspended above the lake bottom were placed in the tubes.

γ-emitting isotopes were added to one of the control tubes on May 27 and to all other tubes on June 17. Each tube received 60 mL of 0.1 N HCl containing a 2 × 10^7 Bq "spike" of each of the following isotopes: ^{203}Hg, ^{65}Zn, ^{59}Fe, ^{51}Cr, ^{60}Co, ^{54}Mn, ^{48}V, ^{228}Th, ^{133}Ba, ^{134}Cs, ^{75}Se, and ^{74}As. The isotope solutions were poured onto the surface of the water, which was then agitated mechanically to promote rapid mixing (Schindler et al. 1980a).

Five days after addition of the isotopes, control-tube water was collected in a polyethylene jug just beneath the surface, and sediment samples were taken from sediment traps in the acidified tube and a control tube. On the 27th day a core sample of the top 2 cm of bottom sediment was collected from one of the control tubes.

Laboratory Work

Sample preparation and fractionation — Water samples were filtered through hot H_2O- and dilute HCl-rinsed 0.45-μm Millipore membranes in order to separate the "dissolved" and "particulate" components. In the Lake 303 experiment, a 5-mL aliquot of the filtered water sample was fractionated on a Pharmacia Sephadex G-25 column 25 cm in diameter using 0.025 M sodium borate (pH 9.1) as the eluant, and 5-mL fractions were taken automatically every 3 min. All other Sephadex separations mentioned hereafter were performed in the same manner. The purpose of using Sephadex chromatography was to separate metal species on the basis of molecular size.

The Lake 303 mud sample was freeze-dried, and 3–4 g portions of it were weighed into screw-cap centrifuge tubes and extracted sequentially with (1) benzene/methanol (1:1), (2) H_2O, and (3) 0.1 N NaOH, in that order, to isolate the bituminous (lipophilic) organic complexes, loosely sorbed ions or water-soluble complexes, and humic complexes, respectively, of the trace metals. Each extraction was accompanied by 15 min of ultrasonification, and was followed by centrifugation, after which the supernate was pipetted out, the undissolved residue was rinsed one or more times, and the rinsings were combined with the original extract. In subsequently mentioned extractions the procedure was the same unless otherwise specified. The NaOH extract was fractionated on a Sephadex G-100 column. Sephadex G-100 was used because it separates sedimentary humic extracts into at least two discrete fractions, a "humic" one and a "fulvic" one (T. A. Jackson unpublished data).

In the Lake 223 experiment, control-tube water was freeze-dried to concentrate the nonvolatile trace elements. A 40.1-mg portion of the freeze-dry residue (representing ≃ 2240 mL of water) was redissolved in 10 mL of water, which was divided into three 3-mL portions. To each portion was added 3 mL of (1) H_2O, (2) 1 N $CaCl_2$ (pH 7),

or (3) 1 N HCl (pH 0.1), and the three solutions were dialyzed against deionized distilled water in a Pope multiple dialyzer using cellulose dialysis tubing with a 4.8 nm pore diameter nominally capable of retaining substances of molecular weight $\geq$ 12 000. The purpose of this experiment was to determine (1) to what extent the "dissolved" trace elements were bound to colloids (nondialyzable substances) and (2) to what extent the colloid-bound fraction could be dissociated from these colloids by neutral salts and strong acids.

A 10.00-g (wet weight) portion of the control-tube core sample was sequentially extracted with (1) benzene/methanol (1:1), (2) H_2O, and (3) 0.1 N NaOH, and another 10.00-g portion was sequentially extracted with (1) H_2O, (2) 1 N $CaCl_2$, and (3) 1 N HCl. Ultrasonification was used in the first extraction scheme but not the second. $CaCl_2$ was used to dissolve weakly sorbed, exchangeable forms of the elements (and cations capable of forming soluble chloride complexes), whereas the HCl was intended to dissolve more strongly bound but acid-labile forms such as cations adsorbed by hydrated oxides or complexed by readily protonated ligands of complexing agents. In addition, separate 4.0-g samples of fresh, wet ooze from the sediment traps in the acidified tube and one of the control tubes were extracted with 0.1 N NaOH and 0.1 N HCl in an ultrasonic vibrator. Finally, 5.9374 g (wet weight) of the control-tube ooze and 8.6264 g (wet weight) of ooze from the acidified tube were each extracted with 20 mL of 0.1 N NaOH, and the extracts were fractionated on a Sephadex G-25 column.

Analytical methods — The mud and water samples, and fractions thereof, were analyzed for γ-emitting isotopes with a Princeton Gamma Tech lithium-drifted germanium detector connected with a Canberra 8100 pulse-height analyzer and Hewlett-Packard 9830 computer. All solutions and solid samples were transferred to plastic petri dishes 47 mm in diameter and dried prior to radionuclide analysis. A very thin ($\leq$1 mm), uniform coating was obtained, minimizing errors related to sample geometry and self-absorption. Systematic errors of this kind are estimated to be <2%. Error associated with the random occurrence of the disintegrations and with background correction was estimated by the computer for each spectrum individually; the values ranged from 2 to 6% depending on the number of counts. All isotope activity data are given in counts per minute (cpm) corrected for radioactive decay that occurred after introduction of the nuclides into the water. A more complete treatment of the counting procedure is given by Hesslein et al. (1980).

The Sephadex fractions and solvent extracts were also analyzed chemically and spectrophotometrically. Organic carbon (org. C) was determined by Beckman-915 and -215A analyzers, and ultraviolet (UV) and visible absorption spectra were obtained with a Cary-14 spectrophotometer. In addition, the solutions were wet-ashed with H_2SO_4 and H_2O_2 under UV radiation (Stainton et al. 1974), and then analyzed colorimetrically for total iron (Fe) by the *o*-phenanthroline method (Sandell 1959), for phosphorus (P) by the molybdate method (Stainton et al. 1974), and for nitrogen (N) by the chloramine-T method (Banoub 1972). Sulfur (S) was estimated by wet-ashing with 30% H_2O_2 at 60°C for 1 h followed by turbidimetric determination as $BaSO_4$.

In the spectrophotometric analyses, absorbance (A_λ) was measured at selected wavelengths (λ) for semiquantitative estimation of dissolved humic matter (Schnitzer 1971) (with the understanding, however, that scattering of light by dispersed hydrated ferric oxide and other colloids could have contributed to the "absorbance" readings). In this report, the parameter "A_{465nm}" or "A_{320nm}" invariably represents a monotonic absorption band that increases progressively with decreasing wavelength across the entire UV-visible range; absorption bands of this kind are typical of humic and fulvic acid solutions (Schnitzer 1971). However, "A_{265nm} (peak)" represents a discrete absorption peak at $\simeq$265 nm superimposed on the monotonic band.

The relative preferences, or "affinities," of the metal isotopes for metal-binding agents of the water and sediment were expressed as the percentages of the isotopes concentrated in particular operationally defined fractions (particulate fraction of water, NaOH-extractable fraction of sediment, etc.) or as half the time required for disappearance of the metal from the water column ("half-time for removal"). (The data on affinity for suspended particles and the removal half-time data were taken from Schindler et al. 1980a.) All of these data were then plotted against various characteristic metal properties, including electronegativity (the values of Allred, as recorded by Douglas and McDaniel 1965), ionic radius (Ahrens 1952), and — in the case of transition metals — crystal field stabilization energy (CFSE) (Douglas and McDaniel 1965). The oxidation states tentatively assigned to the metal ions were those deemed most probable under the circumstances (Cotton and Wilkinson 1972), and the choice of CFSE data was based on the assumption of a "strong" ligand field and octahedral coordination, which was considered a likely configuration for the metals investigated (Cotton and Wilkinson 1972). The validity of our assumptions about the values for CFSE and ionic radius may to some extent be judged by how well these values correlate with our empirical research data. Regression lines, correlation coefficients (r), and significance probabilities (P) were computed for the plots by standard statistical procedures.

A subsample of the freeze-dried Lake 303 mud was homogenized in a ball mill and then characterized chemically and mineralogically. Org. C and N were determined by Carlo-Erba CHN analyzer, and total iron (Fe) and manganese (Mn) were estimated by wet-ashing with hot $HClO_4$/HNO_3 (1:3) followed by analysis with a Perkin-Elmer 403 atomic absorption spectrophotometer. Mn and Fe oxides were determined by sequential extraction using $NH_2OH \cdot HCl/HNO_3$ (Chao 1972) followed by citrate/dithionite/bicarbonate (CBD) (Holmgren 1967), and the extracts were analyzed for Mn and Fe by atomic absorption. A portion of the mud was digested with hot 50% H_2O_2 and analyzed by Philips X-ray diffractometer employing Co$K\alpha$ radiation. The sediment samples from Lake 223 were used up in the isotope extractions, and so their chemical and mineralogical composition could not be determined.

Results

Lake 303 Experiment

In the Sephadex G-25 fractionation of filtered lake water collected 44 h after addition of the isotope "spike" (Fig. 1), ^{203}Hg gave one principal peak in the "void" fraction (no. 23) (signifying that it was bound to "high molecular weight" substances, or "colloids," with a nominal molecular weight of >5000 based on calibration with peptides and globular proteins), together with

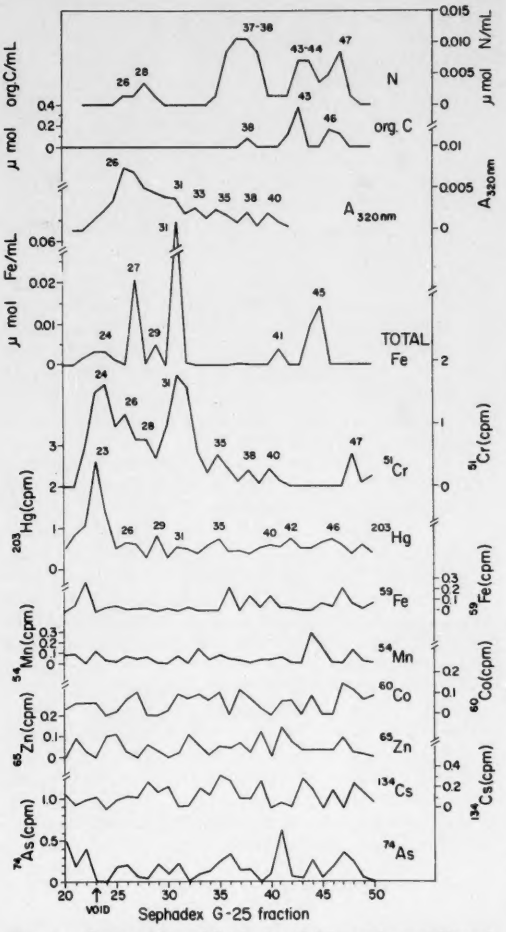

FIG. 1. Composition of Sephadex G-25 fractions of filtered Lake 303 water 44 h after "spiking" with labeled trace elements.

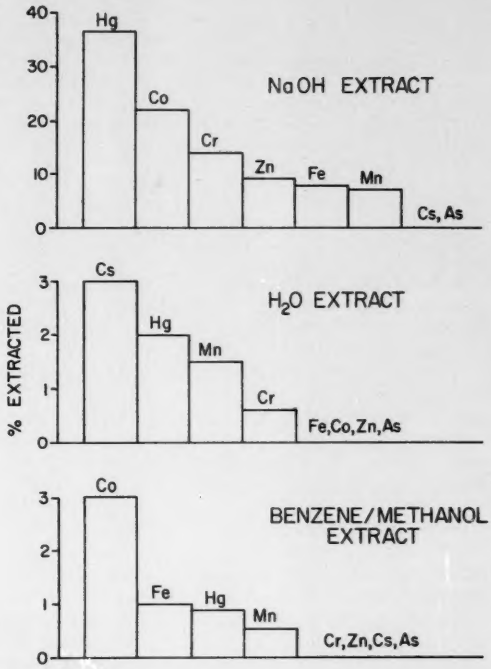

FIG. 2. Percentages of labeled trace elements extracted by benzene/methanol, H_2O, and 0.1 N NaOH from bottom sediment dredged from Lake 303 limnocorral 14 d after "spiking" the water.

several much smaller (possibly insignificant) ones in the lower molecular weight fractions. ^{51}Cr gave two major peaks, one just beyond the void position, and another of lower molecular weight (in fraction 31) plus a number of smaller ones. The other isotopes, whose levels of γ-ray activity were much lower, merely gave a succession of small and apparently insignificant peaks suggesting background "noise."

The principal ^{203}Hg peak and two principal ^{51}Cr peaks coincide with peaks in the distribution of total Fe, but no org. C was detected in the fractions containing these peaks, and the A_{320nm} values, which might otherwise be interpreted as representing humic matter, are evidently due to scattering or absorption of light by inorganic substances containing Fe. Several of the minor ^{51}Cr peaks appear to be associated with N species.

Sequential extraction of the bottom sediment with benzene/methanol, H_2O, and 0.1 N NaOH after 14 d in contact with "spiked" lake water yielded the results shown in Table 1 and Fig. 2. The benzene/methanol, H_2O, and NaOH extracts were green, pale yellow-brown, and deeper brown, respectively. The sediment-bound radionuclides were only 4–37% extractable, and most of the extractable portion was concentrated in the NaOH, or "humic," fraction, except for ^{134}Cs which was concentrated in the aqueous extracts. ^{74}As was not appreciably extracted by any of the solvents, but the results are problematic owing to the low level of ^{74}As in the mud.

The order of decreasing extractability of the radionuclides is quite different for each of the three extractants (Fig. 2). The percentage solubilized by NaOH gave a highly significant positive correlation with the electronegativity of the metal and was therefore greatest for Hg (Fig. 3A); but the percentage extracted by H_2O gave a strong positive correlation with the ionic radius of the metal (Fig. 3B), and the extractability of transi-

TABLE 1. Total γ-ray counts for isotopes in Lake 303 sediment and in benzene/methanol, H_2O, and 0.1 N NaOH extracts and unextracted residue of the sediment. Data are in counts per minute corrected for radioactive decay since the start of the experiment.

Fraction	Hg	Cr	As	Cs	Mn	Fe	Zn	Co
Benz./MeOH	15	0	0	0	10	5	0	5
H_2O	35	7	0	7	28	0	0	0
NaOH	632	177	0	0	136	41	6.8	34
Residue	1094	1062	6	227	1733	484	68	104
Unextracted mud	1726	1265	7	237	1875	517	74	156

tion metals (Co, Fe, Mn, and Cr) by benzene/methanol increased with increasing CFSE (Fig. 3C). Hg was the only nontransition metal detected in the benzene/methanol extract.

The Sephadex G-100 data for the NaOH extract of the sediment revealed wide differences as well as similarities in the distribution patterns of the various nuclides Fig. 4). ^{203}Hg and ^{54}Mn each gave a single major maximum in the void position (fraction 17) (signifying a nominal molecular weight of >150 000), and little activity in lower molecular weight fractions. The major ^{51}Cr peak also occurs in the void fraction but appears to consist of two poorly resolved peaks occurring, respectively, in fractions 17 and 18. As with the lakewater eluate, considerable quantities of ^{51}Cr are found in lower molecular weight fractions as well. Indeed, comparison of Figs. 1 and 4 demonstrates that the Sephadex eluates of the lake water and the NaOH extract of the sediment are similar as far as ^{203}Hg and ^{51}Cr are concerned. ^{59}Fe gave a peak in fraction 17, but the largest peak occurs in fraction 19, whereas ^{54}Mn, ^{51}Cr, and ^{203}Hg show, at most, a small shoulder at or near fraction 19. In contrast to the other metals, ^{65}Zn was chiefly concentrated in relatively low molecular weight fractions, the principal maximum occurring in fraction 27, and only a very minor peak occurred near the void position (in fraction 18). ^{74}As, too, gave only a small peak in the void fraction, and a number of larger ones in lower molecular weight fractions. The distribution patterns of ^{60}Co and ^{134}Cs have the appearance of random noise. Note that up to fraction 28 the Group IIB metals Zn and Hg show nearly the same qualitative distribution pattern but differ strikingly in the proportions of the nuclides partitioned among the different fractions, Hg displaying a pronounced higher-molecular-weight preference.

The principal ^{59}Fe peaks in fractions 17 and 19, and ^{203}Hg, ^{54}Mn, and ^{51}Cr peaks in fraction 17, together with certain minor peaks of these nuclides, and the important ^{65}Zn peak in fraction 27 coincide with peaks for A_{265nm} (peak) and org. C; the ^{65}Zn peaks in fractions 23 and 32–36, however, correspond with minor peaks in the org. C distribution but not with A_{265nm} (peak) maxima. On the other hand, the ^{65}Zn and ^{51}Cr peaks in fraction 18 are associated with major peaks in the distribution of total Fe, N, and A_{465nm} values. The radionuclides in fractions 17 and 18 are also associated with S, which forms a maximum that is spread equally over the two adjacent fractions. A_{265nm} (peak), A_{465nm}, and N all show highly bimodal patterns of variation, with much higher values in the lower molecular weight region, but the Hg, Mn, Fe, and Cr radionuclides, and the total Fe, are preferentially associated with the higher molecular weight material.

The bottom sediment from which the extracts were taken was a mixture of medium-brown mud and fine

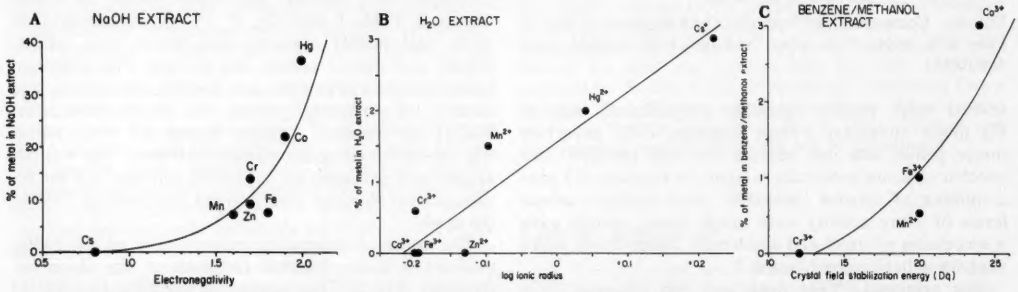

FIG. 3. Relationship between trace metal properties and the percentages of labeled trace metals released from Lake 303 sediment by solvent extractions. A, % extracted by 0.1 N NaOH plotted against electronegativity (semi-log plot; r = 0.967; $P < 0.001$); B, % extracted by H_2O plotted against ionic radius (semi-log plot; r = 0.933; P = 0.001–0.01); C, % extracted by benzene/methanol plotted against crystal field stabilization energy, on assumption of octahedral coordination in "strong field" (semi-log plot; r = 0.917 P = 0.05–0.1).

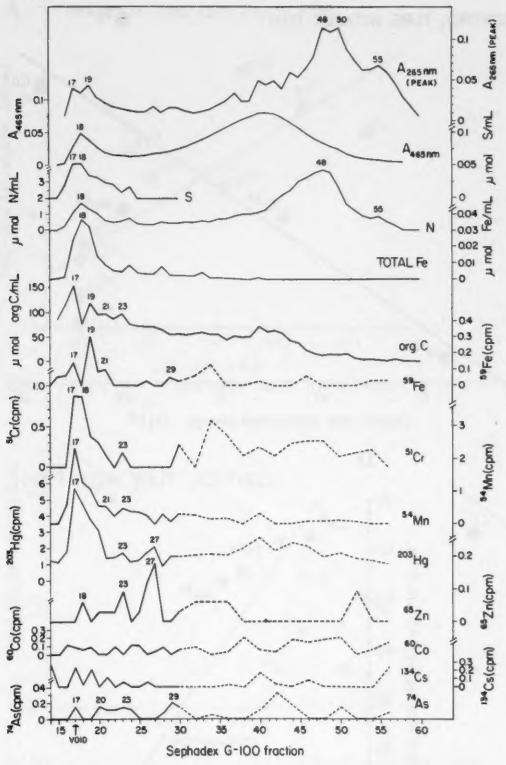

FIG. 4. Composition of Sephadex G-100 fractions of the 0.1 N NaOH extract of Lake 303 sediment collected 14 d after introduction of labeled trace elements.

sand. It was rich in organic matter (24.9% org. C on the basis of dry weight at 105°C and contained appreciable quantities of Fe and Mn oxides (Table 2). An amount of 86.5% of the org. C was in the form of 0.1 N NaOH-extractable "humic" matter, whereas 45.8% of the Fe and ≃100% of the Mn were in the form of extractable "oxides." The principal detrital minerals were quartz and feldspars, but no clay minerals were detected.

LAKE 223 EXPERIMENT

In the water of the control tube without fish, the affinities of metals for >0.45 μm seston, averaged over the first 20 d, increased with the electronegativity of the metal in the case of the nontransition metals (Hg, Zn, Th, Ba, and Cs) (Fig. 5A) and increased with CFSE in the case of the transition metals (Fe, Co, Mn, Cr, and V) (Fig. 5B). In control-tube water containing suckers, the same relationship between transition-metal behavior and CFSE was observed (Fig. 5C); but uptake of nontransition metals by particulate matter is more strongly correlated with ionic radius than with electronegativity, and affinity for the particulate fraction increases with decreasing radius (Fig. 5D). Inspection of the tubes on the 5th day of the experiment revealed greater turbidity in the tube containing fish.

In the acidified tube, no significant correlations between metal properties and preference for particulate matter were found. Nevertheless, all metals — transition and nontransition alike — in the acidified tube showed a significant positive correlation between ionic radius and half-time for removal from the water column (Fig. 6A). In control-tube water containing fish, however, the half-time for removal gave a significant negative correlation with the electronegativity of the metal (Fig. 6B), and the transition metals gave a negative correlation between removal half-time and CFSE (Fig. 6C). In the control tube without fish most of the metals showed a highly significant increase in rate of removal with (1) increasing ionic radius (Fig. 7A) and (2) decreasing electronegativity (Fig. 7B). In both plots Cr has an anomalously long half-time of removal, and in the plot against electronegativity Hg has an anomalously long half-time as well.

When reconstituted water from a control tube containing fish was dialyzed, ^{203}Hg, ^{60}Co, and ^{75}Se were the only radionuclides detectable in the nondialyzable (colloidal) fraction (Table 3). $CaCl_2$ and, to a greater extent, HCl caused some "low molecular weight" ^{203}Hg

TABLE 2. Total and solvent-extractable Fe, Mn, org. C, and N concentrations in bottom sediment from Lake 303 limnocorral, based on oven-dry (105°C) weight. Each number is the mean of two replicate values. ["CDB" means citrate/dithionite/bicarbonate.]

	μmol/g			
		Extractable fraction		
Element	Total	$NH_2OH \cdot HCl/HNO_3$	CDB	0.1 N NaOH
Org. C	20 700	—	—	17 900
N	1940	—	—	—
Fe	149	6.11	62.1	—
Mn	2.6	0.655	2.50	—

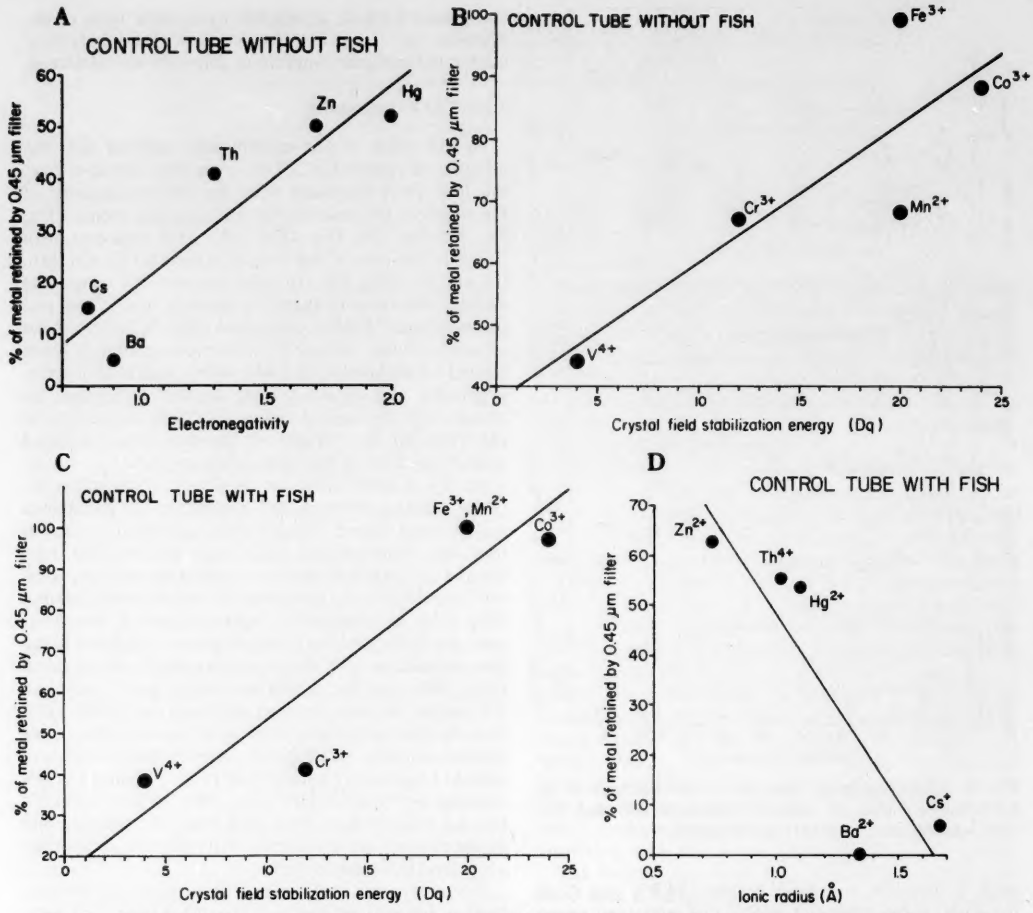

FIG. 5. Relationship between trace metal properties and the proportions of labeled trace metals scavenged by > 0.45 μm suspended particles (averages for the first 20 d) in Lake 223 "control-tube" water in the presence and absence of fish. A, Proportion in > 0.45 μm fraction of control without fish plotted against electronegativity ($r = 0.923$; $P = 0.02$–0.05). [Nontransition metals only]; B, Proportion in > 0.45 μm fraction of control without fish plotted against crystal field stabilization energy, on assumption of octahedral coordination in "strong field" ($r = 0.848$; $r = 0.05$–0.1). [Transition metals only]; C, Proportion in > 0.45 μm fraction of control with fish plotted against crystal field stabilization energy, on assumption of octahedral coordination in "strong field" ($r = 0.914$; $P = 0.02$–0.05). [Transition metals only]; D, Proportion in > 0.45 μm fraction of control with fish plotted against ionic radius ($r = -0.877$; $P \geq 0.05$). [Nontransition metals only.]

and ^{75}Se to be dissociated from the colloids and lost through the dialysis membrane, but even with HCl treatment only about 40% of the colloidal ^{203}Hg and 20% of the colloidal ^{75}Se were lost. ^{60}Co, however, behaved very differently: HCl caused *all* of the colloidal ^{60}Co to be lost, whereas $CaCl_2$ produced an *increase* in the colloidal ^{60}Co fraction and also caused some of the originally low molecular weight dissolved ^{51}Cr to become colloidal.

Solvent extraction of sediments from control tubes with fish and from the acidified tube yielded the results shown in Table 4 and Fig. 8. Although the bottom sediments in these tubes consisted chiefly of sand and gravel, the sediment in the control-tube core sample was medium-brown sandy mud, and the seston collected in the sediment traps under both control and acidified conditions was fine, dark brown, presumably organic ooze. The sequential benzene/methanol, H_2O, and

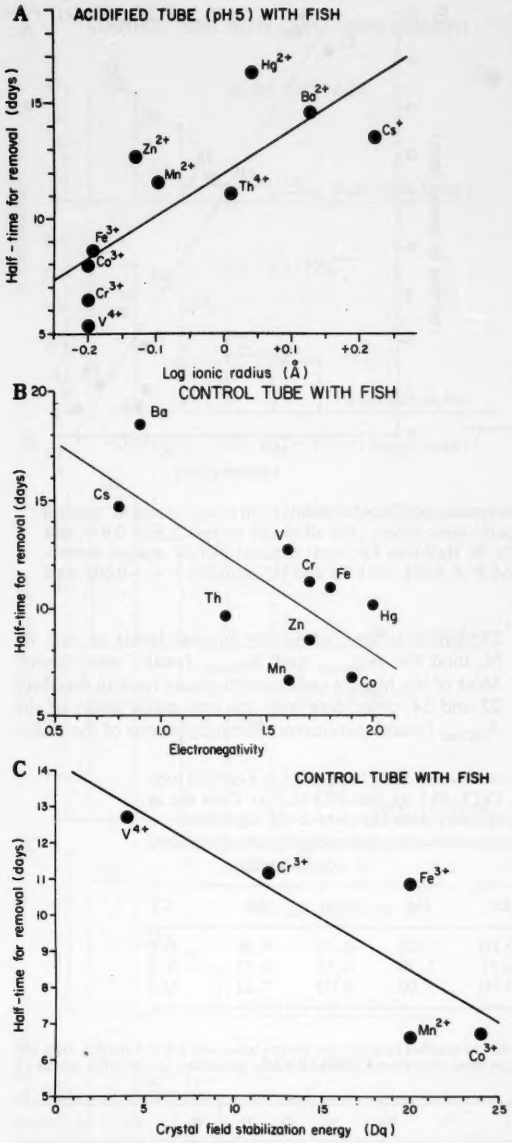

FIG. 6. Relationship between metal properties and rate of disappearance of labeled metals from water column in "acidified tube" and "control tube with fish." A, Half-time for metal removal in acidified tube plotted against ionic radius (semi-log plot; $r = 0.790$; $P = 0.001$–0.01); B. Half-time for metal removal in control tube with fish plotted against electronegativity ($r = -0.731$; $P = 0.01$–0.02); C, Half-time for metal removal in control tube with fish plotted against crystal field stabilization energy, on assumption of octahedral coordination in "strong field" ($r = -0.823$; $P = 0.05$–0.1). [Transition metals only.]

NaOH extracts of the bottom sediment were green, yellow, and brown, respectively; but in the $H_2O/CaCl_2/HCl$ extraction the H_2O and $CaCl_2$ extracts were colorless, and the HCl extract pale yellow. The NaOH extracts of the sediment-trap samples were brown; the HCl extracts were colorless, but, on neutralization with NaOH, a brown precipitate separated out.

In the sequential extractions of control-tube bottom sediment (Table 4; Figure 8), none of the radionuclides were extractable by benzene/methanol or H_2O, and only ^{133}Ba was extractable by $CaCl_2$. Except for ^{133}Ba, all of the solvent-extractable nuclides were extracted solely by NaOH or HCl solution.

The analytical results for the NaOH and HCl extracts of sediments from the acidified tube and from control tubes (both bottom mud and sediment-trap ooze) reveal some interesting regularities as well as differences (Table 4; Fig. 8). ^{203}Hg and ^{75}Se were extractable by NaOH but not by HCl, whereas ^{65}Zn, ^{54}Mn, ^{60}Co, ^{59}Fe, and ^{134}Cs were chiefly or exclusively extractable by HCl. ^{51}Cr and ^{74}As were not extractable by either reagent. Although the sediment-trap samples from acidified and control water gave broadly similar results with respect to most of the radionuclides, ^{228}Th and ^{48}V behaved quite differently in the two environments: In the control tube, ^{48}V was extractable by NaOH but not by HCl, and ^{228}Th was not extracted by either reagent, whereas in the acidified tube ^{48}V and ^{228}Th were extractable by both reagents, and were, in fact, more soluble in HCl than in NaOH. There were also quantitative differences even among the elements that behaved in a fundamentally similar way in both tubes. For instance, the Hg/Se ratio in the NaOH extract was higher for control-tube sediments than for sediment from the acidified tube.

Acidification of the lake water to pH 5.1 had a marked effect on the composition of the NaOH-extractable fraction of the sediment, as revealed by comparison of the Sephadex G-25 fractions of NaOH extracts from acidified and control-tube ooze (Fig. 9). (Note that the data for the acidified tube were recalculated to represent the same weight of sample material as the data for the control tube.) Both samples gave qualitatively similar results as far as the distribution patterns of ^{203}Hg, ^{60}Co, and ^{75}Se were concerned, but the concentrations of ^{203}Hg and ^{60}Co were much lower in the case of the acidified tube. ^{134}Cs, ^{65}Zn, and ^{54}Mn appeared in the control eluate, but neither isotope was even detected in the eluate of the sample from the acidified tube; yet ^{48}V showed up in the eluate of the acidified tube (albeit in only one fraction) but not in the control eluate. Of all the radionuclides detected in the eluates, ^{75}Se showed the least sensitivity to acidification, the concentrations in the two eluates being quantitatively as well as qualitatively similar. Unfortunately, fraction 24 of the eluate representing the

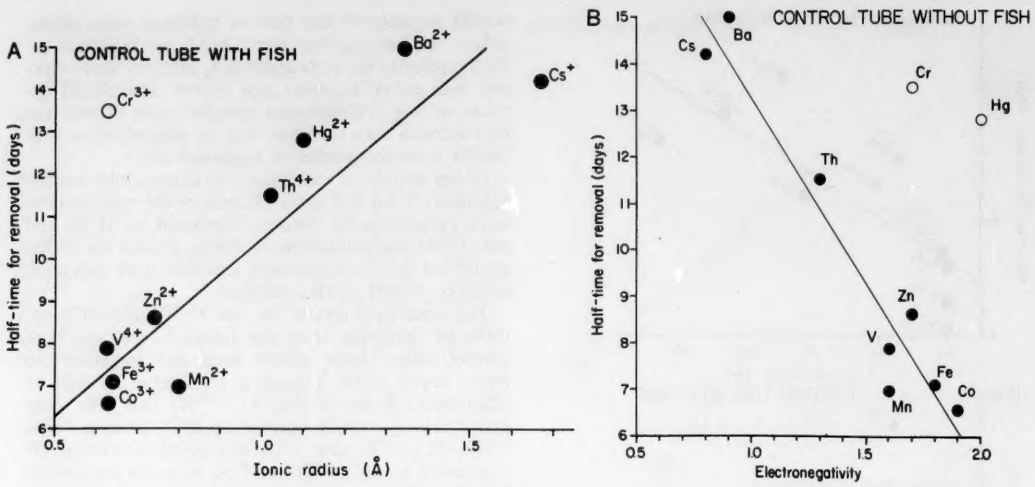

FIG. 7. Relationship between metal properties and rate of disappearance of labeled metals from water column in "control tube without fish." A, Half-time for metal removal plotted against ionic radius. (For all metals except Cr, $r = 0.919$, and $P < 0.001$; with Cr included, $r = 0.721$, and $P = 0.01$–0.02); B, Half-time for metal removal plotted against electronegativity. (For all metals except Cr and Hg, $r = -0.965$, and $P < 0.001$; with Cr and Hg included, $r = -0.602$, and $P = 0.05$–0.1.)

acidified tube was lost, thereby introducing a small element of uncertainty.

Most or all of the ^{203}Hg, ^{60}Co, ^{54}Mn, ^{134}Cs, and ^{65}Zn, and about half of the ^{75}Se, were concentrated in the higher molecular weight Sephadex fractions, 21–28, which is also where the highest levels of org. C, N, total Fe, A_{465nm}, and A_{265nm} (peak) were found. Most of the highest radionuclide peaks were in fractions 22 and 24, coinciding with the two major peaks of the A_{265nm} (peak) parameter. The proportions of the radio-

TABLE 3. Total γ-ray counts for isotopes in reconstituted Lake 223 water from a control tube with fish after dialysis in the presence of H_2O, $CaCl_2$ (0.5 N), and HCl (0.5 N). Data are in counts per minute (cpm) corrected for radioactive decay since the start of the experiment.

	cpm in dialyzate				% nondialyzable			
Fraction	Hg	Co	Se	Cr	Hg	Co	Se	Cr
H_2O	3.93	0.25	8.64	0.00	5.01	0.42	8.86	0.0
$CaCl_2$	2.66	0.31	8.31	0.57	3.39	0.52	8.52	7.8
HCl	2.35	0.00	7.04	0.00	3.00	0.00	7.22	0.0

TABLE 4. γ-ray counts for isotopes in solvent extracts of sediment samples from the acidified tube and two control tubes with fish in Lake 223. Data are in counts per minute corrected for radioactive decay since the start of the experiment. Absorbance values at A_{465nm} normalized to a solution volume of 10 mL are also given.

Sample	Fraction	Se	Hg	Zn	Mn	Co	Fe	V	Cr	Th	Ba	Cs	As	A_{465nm}
Control-tube mud from	Benz./MeOH	0	0	0	0	0	0	0	0	0	0	0	0	—
core	H_2O	0	0	0	0	0	0	0	0	0	0	0	0	0.142
	NaOH	1.57	3.05	0.28	0.75	0.67	0	0	0	0	0	0	0	1.73
Control-tube mud from	H_2O	0	0	0	0	0	0	0	0	0	0	0	0	0.018
core	$CaCl_2$	0	0	0	0	0	0	0	0	0	1.27	0	0	0.010
	HCl	0	0	1.51	9.66	10.5	7.69	0	0	0	0	3.83	0	0.075
Ooze from acidified-tube	NaOH	373	166	0	0	82.8	29.0	101	0	0	0	0	0	2.87
sediment trap	HCl	0	0	41.3	88.3	414	123	209	0	74.8	29.4	93.95	0	0.015
Ooze from control-tube	NaOH	178	298	19.8	28	236	0	107.5	0	0	0	0	0	1.69
sediment trap	HCl	0	0	350	1075	1192	66.6	0	0	0	105	163	0	0.010

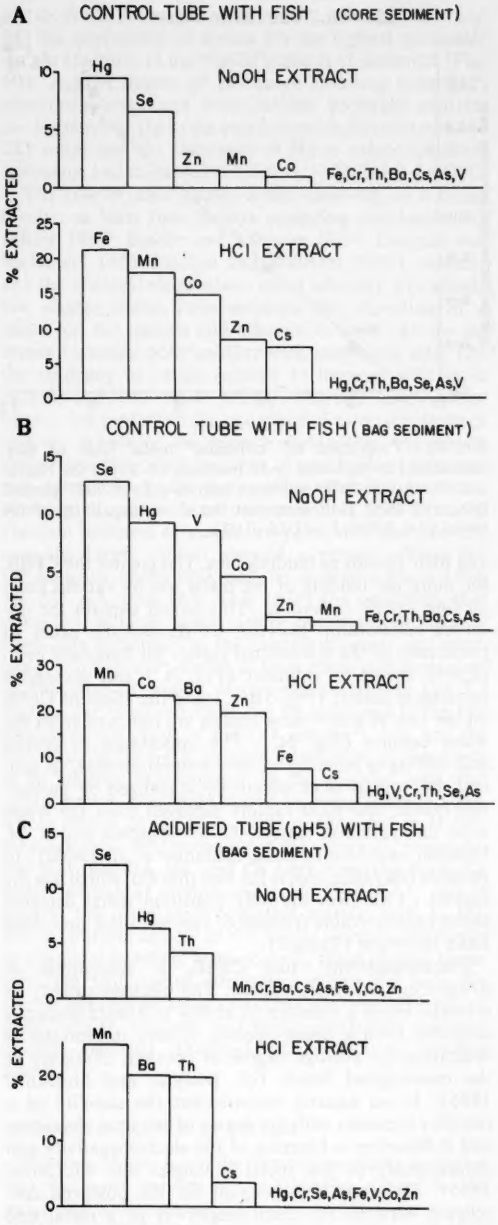

FIG. 8. Percentages of labeled trace elements extracted with NaOH and HCl solutions from sediment samples collected from the "acidified tube" and two "control tubes with fish" in Lake 223.

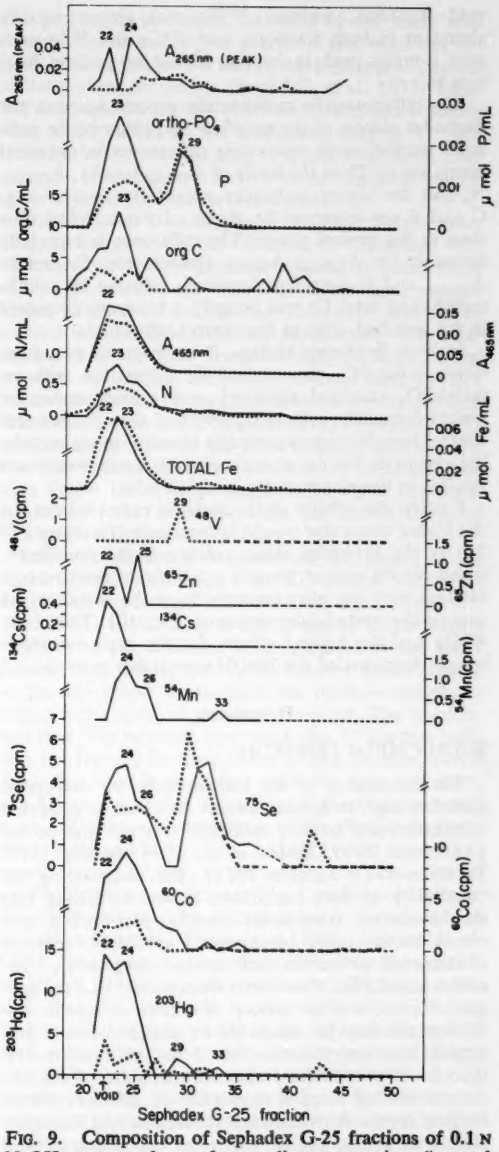

FIG. 9. Composition of Sephadex G-25 fractions of 0.1 N NaOH extracts of ooze from sediment traps in a "control tube with fish" (continuous line) and the "acidified tube" (dotted line) in Lake 223. Breaks in the curve represent missing data. Data were recalculated to represent the same weight of sediment.

nuclides in the two fractions varied, however: ^{60}Co, ^{54}Mn, and ^{75}Se each gave a prominent peak in fraction 24 but no peak or only a shoulder in fraction 22 (the

void fraction), whereas ^{203}Hg was almost equally abundant in both fractions, and ^{134}Cs and ^{65}Zn each gave a major peak in fraction 22 but no peak in fraction 24.

The differences in radionuclide content between the Sephadex eluates of the acidified- and control-tube sediment parallel some interesting differences in chemical composition. Thus, the levels of A_{265nm} (peak), A_{465nm}, N, and the higher molecular weight fractions of org. C and P are lower in the eluate of the acidified tube than in the control eluate. The difference is especially dramatic for A_{265nm}, A_{265nm} (peak), org. C, and P. A_{465nm} and N were less drastically affected by acidification, and total Fe was actually a bit more abundant in the acidified- than in the control-tube eluate.

In both Sephadex eluates, P occurred in two fractions: ortho-PO_4 (as verified by calibration with an ortho-PO_4 standard solution) and a high molecular weight form. The ortho-PO_4 peaks of the acidified and control samples were practically identical in magnitude, but the peak for the macromolecular form was much smaller in the eluate of the acidified tube.

Finally, the affinity of the metallic radionuclides for the higher molecular weight components (fractions 21–28) of the Sephadex eluate relative to the undifferentiated NaOH extract showed a significant positive correlation with the electronegativity of the metal in the case of the control-tube sediment (Fig. 10). Therefore, ^{203}Hg had the highest affinity for the high molecular weight fractions of the NaOH-extractable material.

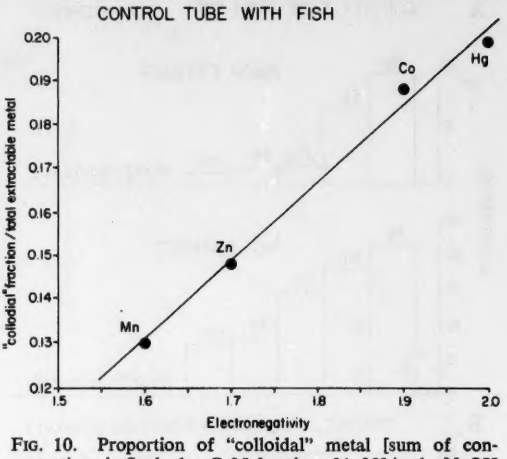

FIG. 10. Proportion of "colloidal" metal [sum of concentrations in Sephadex G-25 fractions 21–28] in the NaOH extract of ooze from sediment trap in a Lake 223 "control tube with fish" plotted against the electronegativity of the metal ($r = 0.996$; $P = 0.01$–0.02).

Discussion

Role of Metal Properties

The scavenging of the radionuclides by suspended particles, high molecular weight dissolved or dispersed substances, and bottom sediments was not unexpected (Andelman 1973; Leland et al. 1974; Brewer 1975; Turekian 1977; Jackson 1978). But, considering the complexity of field conditions, it was surprising that the kinetics of trace-metal binding, partitioning, and remobilization could be expressed as simple functions of elemental properties such as electronegativity, ionic radius, and CFSE. Our results demonstrate that the geochemistry of a wide variety of metals in aquatic environments may be amenable to straightforward prediction and interpretation based on the fundamental theories of coordination chemistry in spite of complications arising from a multitude of different metal-binding agents, environmental influences, and biological effects all acting jointly. However, the interpretations presented here must be regarded as working hypotheses rather than firm conclusions until more information is acquired.

What is the significance of the observed relationships between the behavior of the metals in the limnocorrals and the different physico-chemical properties of these metals? The obvious role of CFSE would be to strengthen the bonds between transitional metal ions and their ligands or binding sites. The greater the CFSE, the more the binding of the metal ion by various complexing agents is favored. This would explain the observed relationship between CFSE and the order of preference of the transitional metals for lipophilic complexing agents in sediment (Fig. 3C) and suspended particulate matter (Fig. 5B, C) and the effect of CFSE on the rate at which these metals are removed from the water column (Fig. 6C). The importance of crystal field effects is reflected in the observation that, in general, the metals most effectively scavenged by particulate matter and most rapidly removed from the water were the transition metals (G. W. Kipphut and R. H. Hesslein unpublished data; Schindler et al. 1980a); of possible relevance, too, is the fact that Co, which has the highest CFSE, was the only transition metal detected in the nondialyzable fraction of reconstituted, untreated Lake 303 water (Table 3).

Electronegativity, like CFSE, is susceptible to straightforward interpretation. The electronegativity of a metal, being a measure of ability to attract electrons available from a donor species, is here interpreted as indicating the average degree of covalent character of the metal-ligand bonds (cf. Douglas and McDaniel 1965). In an aquatic environment the stability of a complex increases with the degree of covalent character, and is therefore a function of the electronegativity and polarizability of the metal (Douglas and McDaniel 1965). This may well account for the observed correlation between the electronegativity of a metal and (1) the percentage of the metal extracted from bottom sediment by NaOH (Fig. 3A), (2) the affinity of nontransition metals for particulate matter (Fig. 5A; also unpublished data for Lake 303 given by G. W. Kipphut and R. H. Hesslein), (3) the rate of removal of the

metals from the water column (Fig. 6B and 7B), and (4) the preference of metals for the highest molecular weight fractions in the NaOH extracts of sediment (Fig. 10). A high degree of covalence resulting from high electronegativity and polarizability probably explains the presence of Hg in the nondialyzable fraction of Lake 223 water and the resistance of Hg to extraction from sediments and dispersed colloids by HCl (Table 3 and 4).

The role of ionic radius is less clear-cut, as it could involve at least four factors operating simultaneously (Hunt 1965; Basolo and Johnson 1964; Douglas and McDaniel 1965; Phillips and Williams 1965): namely (1) the classical electrostatic effect whereby a relatively low charge/radius ratio weakens the attraction of a metal ion for ligands and adsorption sites; (2) the increase in cation polarizability with increasing size; (3) the tendency of cation entropy to increase with ionic radius; and (4) steric effects whereby large, bulky ligands are preferentially coordinated to relatively large cations. Whatever the correct explanation, the results were consistent. Each instance in which metal behavior was dominated by the ionic-radius effect involved partitioning of metals between water and solid material (bottom sediment or suspended particles), and in every case the larger cations showed a greater affinity for the aqueous phase than did the smaller cations (Fig. 3B, 5D, 6A, and 7A). This raises the possibility that metal ions of large radius may be preferentially released from lake sediments to the interstitial water and overlying water column under at least some circumstances. In the case of highly electropositive metals like Cs and Ba, the observed effect can be attributed to the low charge/radius ratios of the Cs^+ and Ba^{2+} ions and the correspondingly weak physical adsorption of these ions in an aqueous environment. In the acidified tube, this interpretation may even apply to some extent to Hg, whose degree of ionic character is enhanced in H_2SO_4 solution; this could account for the seemingly incongruous fact that Hg, Ba, and Cs were removed from the water column at comparable rates (Fig. 6A). But in general the more electronegative metals, such as Hg, fail to conform closely to the simple electrostatic model of chemical bonding; in the case of these metals, therefore, the ionic-radius effect may reflect not only the role of the charge/radius ratio but also the influence of polarizability, entropy, and steric constraints on competition between dissolved and particulate binding agents.

Binding and Release of Metals

We may infer from the limited available evidence that the most important metal-binding agents in the water and sediments of the experimental systems were plankton and other organisms, humic matter and other forms of nonliving organic matter, and hydrated oxides of Fe and Mn; in nature, all of these are avid scavengers of trace metals. The nature of the suspended particles was not determined, but it has been our observation that particulate matter in the lakes of the study area consists almost entirely of plankton and nonliving organic matter; this is reflected in the high org. C content of the bottom sediments (Brunskill et al. 1971; Table 2 of this report). On the other hand, there was probably little or no sulfide in the sediments, judging by their virtual odorlessness and lack of black coloration. Clay minerals, too, appear to have been of negligible significance in the environments investigated.

The Sephadex data for Lake 303 control-tube water (Fig. 1) suggest that the "dissolved" ^{203}Hg and ^{51}Cr were mostly sorbed to peptized colloidal hydrated ferric oxide (represented by total Fe) rather than organic matter (although the oxide may have been peptized by a small amount of sorbed organic matter). The association of some ^{51}Cr fractions with N hints at the presence of ammine complexes of Cr as well; Cr(III) is known to have a strong propensity for combining with ammines (Cotton and Wilkinson 1972). The apparently random distribution of the other isotopes may indicate that the concentrations or activities of these nuclides were below the level of detectability (note that their maximum γ-ray activities were far below those of ^{203}Hg and ^{51}Cr).

Less information is available on the forms of the dissolved radionuclides in Lake 223 control water, but the dialysis experiment provided some clues (Table 3). The relative inefficiency of $CaCl_2$ and HCl in releasing ^{203}Hg from the colloidal binding agents in the water suggests that the Hg was in the form of stable organic — possibly humic or humic-ferric oxide — complexes with a high degree of covalent character. The observation that ^{75}Se behaved very much like ^{203}Hg may indicate the Hg and Se were linked to one another chemically; this idea is consistent with the fact that Se^{2-}, like S^{2-}, has a strong affinity for Hg and other heavy metals (Cotton and Wilkinson 1972). Partial displacement of Hg and Se by $CaCl_2$ and HCl could be ascribed to ion exchange and, in particular, the formation of soluble chloride complexes of Hg. Leaching of Hg from sediments and clay by neutral chloride salts has been reported elsewhere (Feick et al. 1972; Newton et al. 1976). The complete removal of ^{60}Co from the colloids by HCl treatment signifies either that Co, being more ionic than Hg, was more readily displaced by H^+ ions or that the Co was bound to an acid-soluble colloid such as hydrated ferric oxide. The conversion of dialyzable ^{60}Co and ^{51}Cr to nondialyzable forms by $CaCl_2$ treatment could have been caused by flocculation of negatively charged complexes by the "bridging" action of Ca^{2+} ions. The fact that $CaCl_2$ did not have this effect on ^{203}Hg and ^{75}Se suggests partitioning of trace elements among at least two different kinds of binding agent — one (humic matter?) which preferentially complexed Hg and another one (hydrated ferric oxide?) which had a stronger preference for transition metals like Co and Cr.

The relative effectiveness of NaOH and HCl, and the ineffectiveness of H_2O, $CaCl_2$, and benzene/methanol,

in releasing radionuclides from sediment samples constitute evidence that the nuclides were strongly chemisorbed by the sedimentary organic matter and hydrated oxides. The results of the $CaCl_2$ extraction imply that Ba was the only metal which could be liberated from the sediment by a simple cation-exchange process (Table 4).

The NaOH extracts of the Lake 303 and Lake 223 sediments consisted largely of humic matter associated with complexed Fe or peptized hydrated ferric oxide, as is shown by the brown color, UV-visible spectral data, and org. C and total Fe concentrations of the extracts and their Sephadex eluates (Fig. 4 and 9; Table 4). The HCl extracts probably contained appreciable amounts of dissolved ferric oxide, as suggested by the formation of a brown precipitate upon neutralization by alkali; some fulvic acid could also have been solubilized by the HCl. We consistently observed that NaOH extracted Hg and Se preferentially with respect to the other elements (Fig. 2 and 8); on the other hand, HCl extracted greater percentages of transition metals and relatively electropositive nontransition metals such as Cs, Ba, and Zn than did NaOH, but did not extract any Hg or Se. These results probably mean that Hg was bonded to acid-insoluble organic substances such as humic acids by strong, acid-resistant covalent linkages, whereas the other metals were either preferentially sorbed to acid-soluble oxides or were more readily displaced from various ligands and adsorption sites by H^+ ions because their bonds had less covalent character. And, again, the similar behavior of Hg and Se points to a possible chemical linkage between Hg^{2+} and Se^{2-}. Thus, the sediment extractions tended to corroborate the results of the dialysis experiment involving control-tube water.

The Sephadex data for both lakes strongly imply that the NaOH-extractable radionuclides were mostly complexed with the higher molecular weight forms of a group of organic — possibly aromatic — substances yielding an absorption maximum at $\simeq$265 nm. Although these substances have not yet been identified, their characteristic absorption peak is commonly seen in the UV spectra of sedimentary and aquatic humic matter and "yellow substances" produced by aquatic algae (Fogg and Boalch 1958; Yentsch and Reichert 1962; Craigie and McLachlan 1964; Sieburth and Jensen 1968; Jackson and Schindler 1975; T. A. Jackson, unpublished data on UV spectra of humic matter from inland waters and sediments), and similar spectra are given by "green humic acid" (Schnitzer and Skinner 1968). More research is needed to determine the nature and provenance of these compounds, but the limited evidence available suggests that they represent a type of humic substance derived from algal organic matter. The evidence for the complexing of radionuclides by the "humic" substances with the absorption band at 265 nm includes (1) the matching of peaks for chemical, spectral, and γ-emitting isotope data in the Sephadex fractions (Fig. 4 and 9) and (2) the similar responses of A_{265nm} (peak), org. C, and the γ-emitting isotopes to acidification of the lake water, as revealed by comparison of Sephadex eluates for sediments from control and acidified tubes (Fig. 9). Other possible binding agents include hydrated ferric oxide and various organic (but not necessarily humic) compounds, including ones with ligands containing N and S (Fig. 4).

The preference of metal nuclides for the higher molecular weight fractions of the NaOH extracts (Fig. 4 and 9), and the fact that the degree of preference increases with the metal's ability to attract electrons and form covalent bonds (Fig. 10), provide additional indirect evidence that the metals were complexed by aromatic organic substances of the "humic" type. Humic matter is thought to contain condensed aromatic components with delocalized π-electrons (Schnitzer and Khan 1972), and the higher the molecular weight the larger the polynuclear aromatic "core" of the molecule, and hence the more delocalized the π-electrons. Consequently, we would expect the π-electrons of the larger molecules to be more readily funnelled into metal ions complexed by the ligands situated at the edges of the aromatic system, especially in the case of relatively electronegative metals like Hg.

The small quantities of Hg, Mn, and Cr extracted from Lake 303 sediment by H_2O (Fig. 2) may have been in the form of fulvic acid complexes or ions adsorbed to dispersed hydrated ferric oxide, as suggested by the brownish color of the extract, although the Cs extracted could have been in the form of dissolved cations. However, solubilization of metals by benzene/methanol (Fig. 2) can be ascribed mainly to lipophilic organic complexing agents. Degraded chlorophylls were probably among the more important complexing agents, as indicated by the deep green color of the extract and the well-known propensity of porphyrin compounds for chelating metals. Methyl-Hg and elemental Hg as well as coordination complexes are among the possible lipophilic forms of Hg.

Effects of Acidification

Acidification of Lake 223 water produced some interesting effects, as did the activities of fish, but further work is needed to explain them. The fact that acidification appeared to interfere with the uptake of most radionuclides by suspended particles (Schindler et al. 1980a) could reflect competition between H^+ ions and metal cations for binding sites, and the failure of acidification to have any effect whatever on Hg uptake is probably further evidence for the formation of strong, acid-resistant covalent bonds between Hg and the various metal-binding agents in the environment (compare results of HCl extraction of sediments [Table 4]). Again, Hg and Se behaved in a similar manner (Schindler et al. 1980a), suggesting that they were linked to each other chemically (compare Fig. 8). Unexpectedly, Schindler et al. (1980a) observed that lowering the ambient pH actually enhanced the accumulation of Cr and V by

suspended particulate matter. This result was probably not fortuitous in view of the similarities and close kinship between the two elements. Possibly some specific effect of pH on the stability of ligand-cation bonding is involved here.

Considering that acidification of the water did not alter the affinity of Hg for suspended particles, it may seem incongruous that the removal of Hg from the water column was slowed down considerably under acidified conditions (Schindler et al. 1980a). The implication seems to be that the acid interfered with the direct uptake or retention of soluble Hg species by bottom sediments. This interpretation is confirmed by the Sephadex data for NaOH extracts of sediments from acidified and control tubes (Fig. 9): The lowered concentrations of ^{203}Hg and other radionuclides in the acidified samples correspond with lowered concentrations of the "humic" complexing agents with the absorption maximum at 265 nm. Yet, once the Hg was assimilated into the sediment it could not be leached out by HCl (Table 4). The results, then, suggest that Hg may be deposited in sediments as "humic" complexes whose assimilation into bottom sediment was, in this case, inhibited by acidification. Enhanced production of water-soluble CH_3Hg^+ ions is another possible reason for the lowered efficiency of Hg accumulation by sediments under acidified conditions (Jernelöv and Lann 1973; Brouzes et al. 1977).

Another noteworthy result of lake-water acidification was the reduction in the quantity of high molecular weight PO_4 in the sediment (Fig. 9). Possibly hydrolytic cleavage by H^+ ions was involved (Stainton 1980). Potentially available ortho-PO_4 could be released from sediments to the water column by this process. There are grounds for suspecting that the high molecular weight PO_4 consists of PO_4 fixed by Fe and Al complexes of humic matter (Jackson and Schindler 1975; Jackson and Hecky 1977, 1980).

The phenomena treated in this report could have important ecological implications, and therefore warrant more detailed study. Formation of extremely stable, acid-resistant covalent bonds between high molecular weight complexing agents and toxic metals such as Hg may lower the bioavailability and toxicity of these metals. On the other hand, the availability of beneficial micronutrients could also be diminished. Acidification of soft-water lakes would probably not result in appreciable displacement of Hg^{2+} ions from organic bottom sediments by the action of H^+ ions. Nevertheless, acidification could inhibit the transfer of Hg from water to bottom sediments (besides increasing the production of water-soluble monomethyl Hg), and could cause the release of various metals that are more electropositive than Hg, together with PO_4, into the water column, where they might be made biologically available.

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Dynamics of Cadmium-Stressed Plankton Communities[1]

J. S. MARSHALL AND D. L. MELLINGER

Ecological Sciences Section, Radiological and Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA

MARSHALL, J. S., AND D. L. MELLINGER. 1980. Dynamics of cadmium-stressed plankton communities. Can. J. Fish. Aquat. Sci. 37: 403–414.

Structural and functional responses of plankton communities to cadmium stress were studied during 1977 in Lake Michigan using small-volume (8 L) completely sealed enclosures, and in Canada's Experimental Lakes Area (ELA) Lake 223 using large-volume (1.5×10^5 L) open-surface enclosures. In Lake Michigan, reductions of the average abundance of microcrustaceans by cadmium were significantly greater in "light" or shallow epilimnetic incubations than they were in "dark" or deep epilimnetic incubations. Measurements of dissolved oxygen indicated that this interaction with light (depth) was an indirect effect due to a reduction of photosynthesis and primary production. Zooplankton density and species diversity were not significantly affected within 21 d by cadmium concentrations $\leqq 0.6$ and $\leqq 1.2$ μg Cd/L, respectively, whereas final dissolved oxygen concentration and percentage similarity (*PS*) of the crustacean zooplankton community were significantly reduced by $\geqq 0.2$ μg Cd/L. In the ELA Lake 223 experiment, the reducing effect of cadmium on zooplankton density increased up to 31 d after Cd enrichment and then decreased, probably due to decreasing Cd concentrations in the water. Values of *PS* on day 24 for the ELA enclosures enriched with 1 and 3 μg Cd/L were within the 95% confidence limits for individual values predicted from a regression of *PS* on cadmium for the 21-d Lake Michigan experiments.

Key words: plankton communities, zooplankton, phytoplankton, cadmium stress, Lake Michigan, Canadian Shield lakes

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Utilisant des récipients de faible volume (8 L) entièrement scellés dans le lac Michigan et des enceintes de grand volume ($1,5 \times 10^5$ L) à surface libre dans le lac 223 de la région des lacs expérimentaux (ELA) du Canada, nous avons étudié les réactions structurales et fonctionnelles de communautés planctoniques au stress du cadmium. Dans le lac Michigan, la diminution es l'abondance moyenne des microcrustacés due au cadmium est nettement plus grande dans des incubations «de clarté» ou de région épilimnétique peu profonde que dans des incubations «de noirceur» ou de région épilimnétique profonde. Les mesures d'oxygène dissous indiquent que cette interaction avec la lumière (profondeur) est un effet indirect d'une diminution de photosynthèse et de production primaire. La densité et diversité des espèces du zooplancton ne sont pas affectées de façon appréciable en dedans de 21 jours par des concentrations de cadmium $\leqq 0,6$ et $\leqq 1,2$ μg Cd/L respectivement. Par contre, les concentrations finales d'oxygène dissous et le pourcentage de similitude (*PS*) de la communauté zooplanctonique de crustacés diminuent de façon marquée à $\leqq 0,2$ μg Cd/L. Dans l'expérience du lac 223 de la ELA, l'effet réducteur du cadmium sur la densité du zooplancton augmente jusqu'à 31 jours après enrichissement au Cd, pour ensuite diminuer, probablement à cause d'une diminution des concentrations de Cd dans l'eau. Au jour 24, les valeurs de *PS* dans les enceintes de la ELA enrichies de 1 et 3 μg Cd/L se situent en dedans des limites de confiance à 95% pour les valeurs individuelles prédites à partir d'une régression de *PS* sur le cadmium dans les expériences de 21 jours sur le lac Michigan.

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EXPERIMENTAL approaches to limnology have long been in use, but only recently have they been employed extensively to study ecosystem responses to pollutant enrichment and other stresses. Recent experimental studies of aquatic ecosystems have employed laboratory microcosms (Leffler 1978), artificial ponds (Hall et al. 1970), whole lakes (Schindler and Fee 1974), and in situ enclosures of various sizes and types (Gamble et al. 1977; Grice et al. 1977; Kuiper 1977; Lean et al. 1975; Smyly 1976). The usefulness of in situ enclosures in studies of heavy metal effects on plankton communities was shown by Reeve et al. (1976) who concluded that the zooplankton was probably the com-

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ponent most affected. Large enclosures have been used primarily in small lakes or protected areas of larger water bodies. Large polyethylene bags have been used in Lake Michigan in experiments lasting up to 2 wk, but difficulties were encountered due to rigorous physical conditions (Schelske and Stoermer 1972). Therefore, for studies of energy-related pollutant effects in Lake Michigan, we developed an in situ technique using polyethylene carboys as enclosures. The effects of enclosure and in situ incubation for 4–15 d in polyethylene carboys on the zooplankton community in northern Green Bay, Lake Michigan, were studied during 1976 in connection with preliminary cadmium enrichment experiments (Marshall and Mellinger 1978a). This study indicated that the effects of enclosure on the zooplankton populations were relatively minor and that in situ experiments longer than 15 d were feasible.

Cadmium concentrations >5 μg Cd/L in our 1976 in situ experiments caused pronounced effects on the Lake Michigan zooplankton community within 9 d; these experiments also indicated that much less than 5 μg Cd/L would probably cause readily detectable effects (Marshall and Mellinger 1978a). Other studies indicate that zooplankton are more sensitive to cadmium than either phytoplankton or fish and thus may be critical in determining the overall impact of cadmium in lacustrine ecosystems such as Lake Michigan and Canadian Shield lakes. Cadmium concentrations ≧0.2 μg Cd/L are toxic to laboratory zooplankton populations (Biesinger and Christensen 1972), whereas cadmium has not heretofore been reported to be toxic to phytoplankton at <2 μg Cd/L (Conway 1978) or to fish at <3 μg Cd/L (Benoit et al. 1976). Cadmium concentrations potentially toxic to zooplankton (≧0.2 μg Cd/L) probably occur in many contaminated lakes in North America. For example, concentrations >0.2 μg Cd/L occur in several lakes near smelters at Sudbury, Ontario, and Flin Flon, Manitoba, although the concentration of cadmium in 109 ELA lakes is <0.1 μg Cd/L (Beamish and Van Loon 1977; Van Loon and Beamish 1977). Cadmium concentrations reported for Lake Michigan (Leland 1975; Surles et al. 1975) and Lake Ontario (Chau et al. 1970) are ~0.1 μg Cd/L, although work in progress indicates that the cadmium concentration in the offshore waters of Lake Michigan is <0.1 μg Cd/L (G. T. Tisue, Argonne National Laboratory, personal communication).

This paper is concerned primarily with the responses of the zooplankton communities in two freshwater ecosystems, Lake Michigan and ELA Lake 223, to cadmium enrichment in experiments conducted in 1977. Consideration is also given to the responses of the phytoplankton communities to cadmium and the effects of enclosure on the zooplankton. Following guidelines recently recommended for ecosystem stress studies (Barrett et al. 1976), we studied both functional and structural attributes of these ecosystems under seminatural conditions and evaluated the relative sensitivity of different indices of stress.

Materials and Methods

Six experiments were conducted in northern Green Bay, Lake Michigan, during 1977 to determine the effect of cadmium enrichment in the 0–5 μg Cd/L range and its interaction with light (depth). These experiments were conducted in the same location as those in 1976 (Marshall and Mellinger 1978a) — 1 km northwest of the larger Sister Island off Sister Bay, Wisconsin. Water was collected from different depths in the epilimnion with a nonmetallic, 16-L Kemmerer bottle and mixed in a 50-L polyethylene cylinder under the shade of a canvas top on a pontoon boat. In each experiment, duplicate 8-L polyethylene carboys for five concentrations of cadmium at each of two depth ranges were filled with the mixed water. Additional 8-L carboys were filled at the beginning and termination of each experiment for analyses of zooplankton and dissolved oxygen in unincubated lake samples. When all of the carboys were nearly filled, those for in situ incubation were assigned at random to prescribed cadmium ($CdCl_2$) additions: 0, 0.6, 1.2, 2.5, and 5.0 μg Cd/L (nominal concentrations) in experiments 1–4; and 0, 0.2, 0.4, 0.8, and 1.6 μg Cd/L in experiments 5 and 6. Required volumes of a $CdCl_2$ solution were dispensed by serological pipet. Each carboy was then capped, thoroughly shaken, and finally filled completely to exclude all air bubbles. The carboys were suspended at two depth ranges in the epilimnion as indicated in Fig. 1. Experiments 1 and 2 differed from the others in that the shallower set of carboys (at 3–8 m) were opaque, and only the deeper set (9–10 m) were translucent. The six experiments were conducted in three 3-wk incubation periods: July 19–August 9 (experiments 1 and 2); August 10–30 (experiments 3 and 4); August 31–September 21 (experiments 5 and 6). At the termination of the experiments, the concentration of dissolved oxygen in each carboy was measured with a YSI Model 57 Oxygen Meter, and the zooplankton were removed (by filtering through an 85-μm aperture net) and preserved in 4% formalin. Light intensities at different depths, both in the lake and inside a translucent polyethylene carboy filled with lake water, were measured with a Protomatic underwater photometer.

In ELA Lake 223, a single cadmium enrichment experiment was conducted during 1977 using five large enclosures. These were nylon-reinforced polyethylene cylinders, 10 m in diameter, extending from the surface to the bottom at a depth of approximately 2 m. Each cylinder was supported at the surface by a ring of plastic-foam floats and secured at the bottom by weights. On July 4 (day 0), $CdCl_2$ was added to four of the enclosures to produce nominal concentrations of 1, 3, 10, and 30 μg Cd/L, leaving one enclosure as a control. Tritiated water and ^{115m}Cd were also added as radiotracers for water balance and cadmium kinetics studies. After July 4 samples of water, plankton, benthos, fish, and sediments were collected periodically by ELA staff for various analyses. Zooplankton samples were collected with a quadruple tow net (one pair with 153-μm apertures and 15-cm mouth diameters and another pair with 56-μm apertures and 8-cm mouth diameters) at approximately weekly intervals from July 8 until August 17 (day 44) and finally on October 28 (day 87). Composite samples were collected on each date by combining four vertical hauls (bottom to surface) from each enclosure. Samples from the open lake were collected similarly on July 24, August 17, and October 28. The samples col-

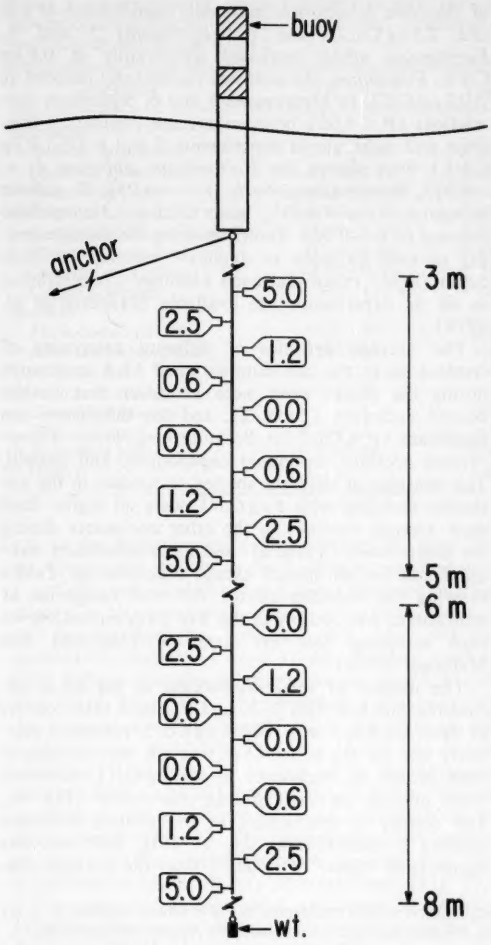

FIG. 1. Diagram of typical in situ incubation arrangement using polyethylene carboys (numbers show added Cd concentrations) as enclosures in Lake Michigan experiments.

lected by the 153-μm and 56-μm nets were preserved separately in 4% formalin.

Zooplankton identifications were made with the aid of keys, illustrations, and descriptions in Edmondson (1959) and Chengalath et al. (1971). Quantitative analyses were made of rotifers and nauplii in a Sedgewick-Rafter chamber and of other crustaceans in a chambered counting cell (Gannon 1971) using Wild M20 and M5 microscopes, respectively. Rotifers and nauplii were counted only in the ELA Lake 223 samples collected with the 56-μm nets. Crustaceans, exclusive of nauplii, were counted in the Lake Michigan and ELA Lake 223 samples collected with the 85-μm and 153-μm nets, respectively.

Three indices of species diversity were calculated for the crustacean zooplankton communities according to Hill (1973):

(1) N_0 = number of identification categories

(2) $N_1 = \exp(-\Sigma p_i \ln p_i)$

(3) $N_2 = 1/\Sigma p_i^2$,

where p_i is the proportional abundance of a given identification category in a sample.

Two similarity indices, coefficient of community (*CC*) and percentage similarity (*PS*), were also calculated for the crustacean zooplankton communities according to Whittaker (1972):

(4) $CC = 2S_s/(S_j + S_k)$,

where S_j is the number of identification categories in the control samples, S_k is the number in any single sample, and S_s is the number common to both; and

(5) $PS = 1 - 0.5\Sigma|p_{ij} - p_{ik}|$,

where p_{ij} is the average proportional abundance of a given identification category in the control samples and p_{ik} is its proportion in any single sample. The calculated values of *CC* and *PS* are multiplied by 100 and expressed in this paper as percentages.

Enclosure effects were assessed from calculations of *CC* and *PS* for the control samples relative to the average proportional abundance composition of the terminal open lake samples and for the individual lake samples relative to the same average lake composition. In these calculations, S_j (eq. 4) was the number of identification categories in the terminal open lake samples, S_k was the number in any single sample (enclosure or lake), and S_s was the number common to both; similarly, p_{ij} (eq. 5) was the average proportional abundance of a given identification category in the terminal lake samples, and p_{ik} was its proportion in any single sample.

Results

Zooplankton Populations

The responses of crustacean species populations to cadmium enrichment in the Lake Michigan experiments included a few population increases at 0.6–2.5 μg Cd/L, as well as general decreases at 5.0 μg Cd/L (Fig. 2). Population densities at each cadmium concentration varied with incubation depth, so paired *t*-tests (Cd-control pairs matched by depth) were used to determine the significance of differences in average density due to cadmium. In experiment 3, for example, paired *t*-tests indicated that the average densities of *Daphnia retrocurva*, *D. longiremis*, *Bosmina longirostris*, and cyclopoid copepodites were significantly higher at 0.6 or 1.2 μg Cd/L than in the controls, although all species populations were lower at 5.0 μg Cd/L (Table 1). *Eubosmina coregoni* (Fig. 2) and *Holopedium gibberum* were significantly reduced at $\geqq$0.6 μg Cd/L. The results for experiment 4 were essentially the same as those summarized for experiment 3 (Table 1). In experiments 1 and 2, the density

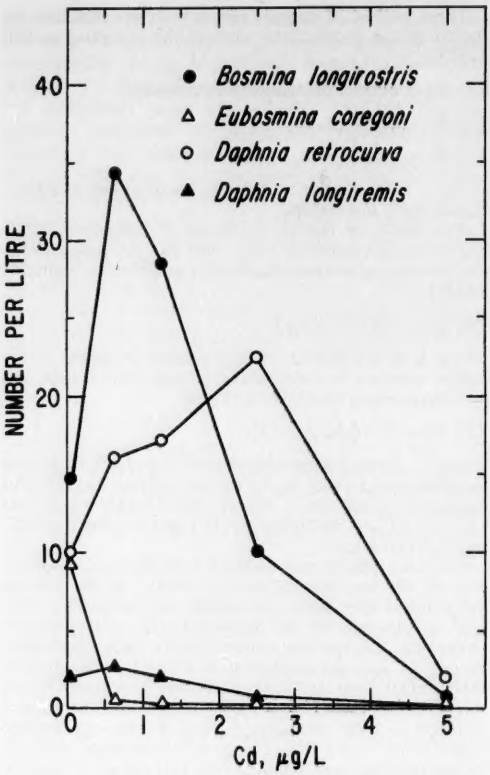

FIG. 2. Typical effects of cadmium on average numerical density of four species of cladocerans, including three that increased significantly at concentrations equal to or less than 2.5 μg Cd/L, in the Lake Michigan experiments. Results shown are from experiment 3.

of *Bosmina longirostris* increased significantly at 1.2 and 2.5 μg Cd/L, and in experiments 5 and 6, *Eurytemora affinis* increased significantly at 0.8 μg Cd/L. *Eubosmina coregoni* was significantly reduced at ≧0.2 μg Cd/L in experiments 5 and 6. Significant correlations ($P < 0.05$) between average population densities and cadmium in experiments 5 and 6 (0–1.6 μg Cd/L) were shown for *Holopedium gibberum* ($r = -0.92$), *Bosmina longirostris* ($r = +0.95$), *D. galeata mendotae* ($r = -0.89$), and *Cyclops bicuspidatus thomasi* ($r = -0.92$). Tables showing the average density of each category in duplicate samples for each depth (light) range and each cadmium concentration in all six experiments are available (Marshall et al. 1978).

The average densities of different categories of crustaceans in the cadmium-enriched ELA enclosures during the fourth week were all lower than in the control enclosure (Table 2), and the differences are significant ($P < 0.05$ for *Bosmina longirostris*, *Tropocyclops prasinus*, cyclopoid copepodites, and nauplii. The densities of different species of rotifers in the enclosure enriched with 1 μg Cd/L were all higher than their average densities in the other enclosures during the fourth week (Table 2), and these differences were significant for all species except *Ploesoma* sp. Tables showing the densities of the different categories of crustaceans and rotifers in the five ELA enclosures on each sampling date are available (Marshall and Mellinger 1978b).

The density of total crustaceans in the ELA enclosures enriched with 3–30 μg Cd/L and total rotifers in those enriched with 10–30 μg Cd/L remained relatively low for the entire 44 d, whereas the densities of both groups in enclosures at 0–1 μg Cd/L exhibited some marked increases during this period (Fig. 3). The density of crustaceans in the control enclosure reached a maximum on day 31 (Fig. 3A) and was significantly higher ($P < 0.05$) than the average den-

TABLE 1. Average density of different categories of crustacean zooplankton in four enclosures at 3–8 m (mean number/L ± SE) for each of five concentrations of added cadmium after incubation in Lake Michigan (Experiment 3, August 10–30, 1977).

Category	0 μg Cd/L	0.6 μg Cd/L	1.2 μg Cd/L	2.5 μg Cd/L	5.0 μg Cd/L
Holopedium gibberum	4.98±2.39	0.03±0.03	0	0	0
Bosmina longirostris	14.65±2.98	34.19±6.76	28.47±7.02	10.15±3.35	0.71±0.30
Eubosmina coregoni	9.28±3.20	0.44±0.04	0.12±0.09	0.19±0.08	0.33±0.11
Daphnia retrocurva	10.15±4.90	16.10±5.88	16.85±5.70	22.60±9.61	1.71±0.61
D. galeata mendotae	5.81±0.15	4.15±1.34	4.40±0.48	5.00±1.42	3.50±1.18
D. longiremis	1.78±0.89	2.35±0.81	1.85±0.46	0.59±0.11	0
Ceriodaphnia lacustris	0,78±0.34	1.22±0.37	1.38±0.34	1.19±0.32	0.54±0.48
Chydorus sphaericus	52.38±12.25	45.12±9.62	39.75±5.25	22.50±1.58	5.88±2.22
Cyclops vernalis	2.81±0.34	2.75±0.26	2.65±0.26	2.25±0.15	2.16±0.46
C. bicuspidatus thomasi	3.50±0.52	2.25±0.45	2.52±0.62	2.60±0.76	1.25±0.15
Mesocyclops edax	1.50±0.20	1.12±0.24	1.28±0.14	1.25±0.20	1.00±0.15
Tropocyclops prasinus mex.	4.31±1.20	4.28±1.38	3.50±1.00	4.60±1.75	3.04±1.18
Cyclopoid copepodites I–V	5.00±0.34	5.75±0.36	5.31±0.31	5.85±0.85	4.25±0.48
Eurytemora affinis	0.75±0.18	0.85±0.15	0.72±0.24	0.85±0.22	0.41±0.24
Diaptomus sp.	0.25±0.10	0.19±0.11	0.40±0.10	0.38±0.08	0.17±0.09
Calanoid copepodites I–V	0.52±0.10	0.56±0.08	0.62±0.19	0.50±0.14	0.16±0.11

TABLE 2. Average density (mean number/L ± SE) of different categories of plankton crustaceans and rotifers in ELA Lake 223 enclosures during fourth week (24–31 d) after enrichment with different nominal concentrations of cadmium. Sampling dates were July 28 and August 4, 1977.

Category	0 μg Cd/L	1 μg Cd/L	3 μg Cd/L	10 μg Cd/L	30 μg Cd/L
Crustacea					
Bosmina longirostris	5.15±1.69	2.47±0.24	0.54±0.04	0.03±0.01	0.06±0.03
Chydorus sphaericus	0.16±0.07	0.10±0.06	0.03±0.01	<0.01	0.48±0.16
Mesocyclops edax	0.02±0.02	0.02±0.01	0.03±0.02	0.01±0.01	<0.01
Tropocyclops prasinus mex.	4.88±1.56	2.21±0.38	0.54±0.03	0.14±0.01	0.04±0.01
Cyclopoid copepodites I–V	10.16±1.65	3.31±1.54	0.77±0.25	0.12±0.01	0.23±0.10
Diaptomus minutus	0.17±0.03	0.07±0.01	0.29±0.11	0.04±0.01	0.01±0.01
Calanoid copepodites I–V	0.84±0.34	0.75±0.10	0.50±0.06	0.09±0.01	0.07±0.05
Copepod nauplii	335±121	30±20	15±5	5±1	13±4
Rotifera					
Trichocerca cylindrica	163±37	219±27	46±4	1±1	1±1
Keratella taurocephala	168±42	314±176	42±12	2±1	10±1
K. cochlearis	0	30±24	13±10	1±1	0
Kellicottia longispina	1±1	3±3	0	0	0
Conochilus unicornis	1±1	147±61	80±35	12±10	21±10
Ploesoma sp.	18±6	14±2	3±3	0	4±2
Polyarthra spp.	224±68	1038±362	116±10	77±12	149±54
Others	4±1	8±4	6±2	4±4	2±0

sity in the cadmium-enriched enclosures on days 17–44. Crustacean density in the enclosure enriched with 1 μg Cd/L did not differ significantly from the average density in those enriched with 3–30 μg Cd/L through day 24, but thereafter increased (Fig. 3A), and was significantly higher on days 31–44. Rotifers reached their highest densities in the enclosure enriched with 1 μg Cd/L (Fig. 3B). They reached a maximum on day 31 and were significantly more abundant in this enclosure than in the others on days 17–44. The decline of rotifers from day 31 to day 44 in the enclosure enriched with 1 μg Cd/L coincides with the increase of crustaceans (potential predators and competitors) in this enclosure (Fig. 3). The consistently lower density of rotifers in the control enclosure also coincides with a consistently higher density of crustaceans (Fig. 3).

The average density of total crustaceans in the Lake Michigan enclosures also decreased with cadmium in the 0–5 μg Cd/L range, and this effect of cadmium was modified by incubation depth (light) conditions. Paired *t*-tests indicated that total crustacean density was significantly reduced by the highest concentration of cadmium used in each of the three experimental periods (Table 3). The lowest concentration causing a significant reduction was 0.8 μg Cd/L in the third period. The linear relationship between density of total crustaceans and cadmium (Fig. 4) predicts that average density would be reduced by 50% at 3.5 μg Cd/L and by 1% at 0.07 μg Cd/L. In toxicological terms, the median effective concentration (EC50) for reduction of relative crustacean abundance (N') is 3.5 μg Cd/L and the EC1 is 0.07 μg Cd/L.

The effect of cadmium on the average density of crustaceans after incubation at different depth (light) ranges in Lake Michigan is illustrated in Fig. 5. The line for 3–5 m is represented by the equation $\bar{N} = 173 - 29.1\,\text{Cd}$ ($r = -0.99$), while that for 6–8 m is represented by $\bar{N} = 87 - 11.2\,\text{Cd}$ ($r = -0.98$). A *t*-test indicated that the difference between the slopes is highly significant ($P < 0.001$). Similarly, the slope for translucent enclosures was greater than that for opaque enclosures in both of the first two experiments, and the slope for translucent enclosures at <6 m was greater than that for enclosures at >6 m in each of the other experiments. The effect of cadmium on total crustacean density ($\bar{N}$) thus appears to be intensified by light because the slopes of regressions of $\bar{N}$ on Cd

TABLE 3. Average density of total crustaceans (excluding nauplii) in enclosures at each added cadmium concentration after in situ incubations in Lake Michigan during three periods in 1977 (mean ± SE).

Period	Cd μg/L	Density animals/L
July 19–Aug. 9	0	40±10
	0.6	28±6
	1.2	27±7
	2.5	20±3
	5.0	8±1
Aug. 10–30	0	112±15
	0.6	121±14
	1.2	116±13
	2.5	94±15
	5.0	31±6
Aug. 31–Sept. 21	0	72±7
	0.2	67±9
	0.4	62±7
	0.8	56±5
	1.6	59±8

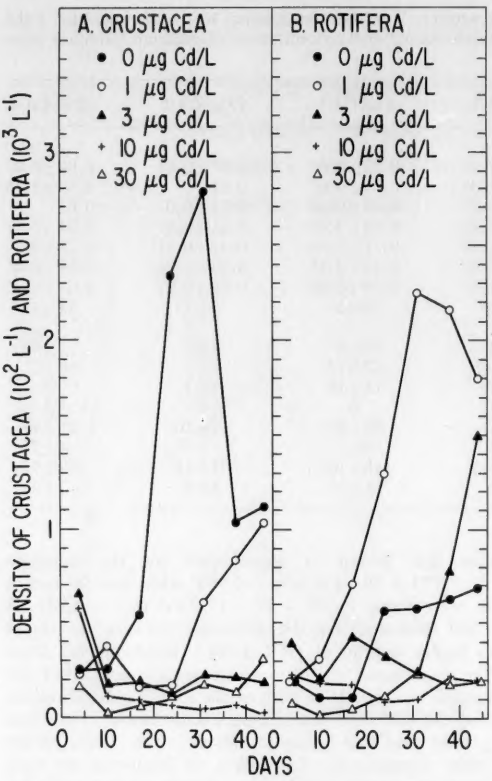

FIG. 3. Temporal variations in numerical density of planktonic crustaceans and rotifers in ELA Lake 223 enclosures initially enriched with different concentrations of cadmium.

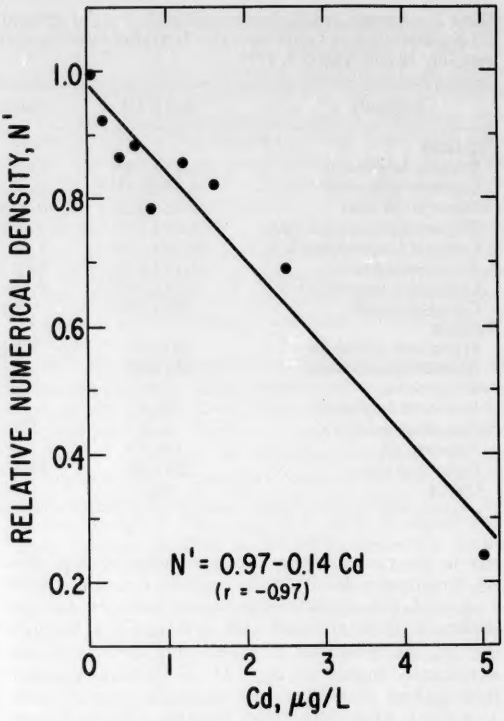

FIG. 4. Effect of cadmium on relative numerical density (fraction of control density) of planktonic crustaceans, exclusive of nauplii, in Lake Michigan enclosures.

were consistently greater (steeper) under the conditions of higher light intensity. (The vertical extinction coefficient calculated from measurements of light intensities inside a translucent enclosure at different depths was the same as that outside, but the intensity of light inside at each depth was equal to that about 1.7 m deeper on the outside.)

ZOOPLANKTON COMMUNITY STRUCTURE

Species diversity, as indicated by Hill's numbers (N_0, N_1, and N_2), was relatively insensitive to cadmium in both ELA Lake 223 and Lake Michigan. The lowest cadmium concentration causing a significant reduction of N_0 in the Lake Michigan experiments was 2.5 μg Cd/L during July 19–August 9 (Table 4). A significant reduction of N_0 at 5 μg Cd/L in both of the first two periods (Table 4) is consistent with the results of our previous study (Marshall and Mellinger 1978a). The increases in N_1 and N_2 (Table 4) are due to the evenness component of diversity because species richness was reduced. Increased evenness was also indicated by an increase in the N_2/N_1 ratio (Hill 1973).

Both indices of community similarity, coefficient of community (*CC*) and percentage similarity (*PS*), responded more consistently to cadmium than did indices of species diversity (Hill's numbers), although *PS* was more sensitive than *CC*. In the Lake Michigan experiments, *PS* was significantly reduced by ≧1.2, ≧0.6, and ≧0.2 μg Cd/L in the 1st, 2nd, and 3rd periods, respectively, whereas *CC* was not significantly reduced by <2.5 μg Cd/L (Table 5). A linear relationship between *PS* and cadmium is represented by the regression equation and line fitted to the average values of *PS* for the Lake Michigan experiments (Fig. 6). The values of *PS* for the ELA Lake 223 enclosures enriched with 1 and 3 μg Cd/L on day 24 are within the 95% confidence intervals for individual values of *PS* predicted from the regression for the 21-d Lake Michigan experiments. The ELA Lake 223 *PS* values for day 31 also fall within these 95% con-

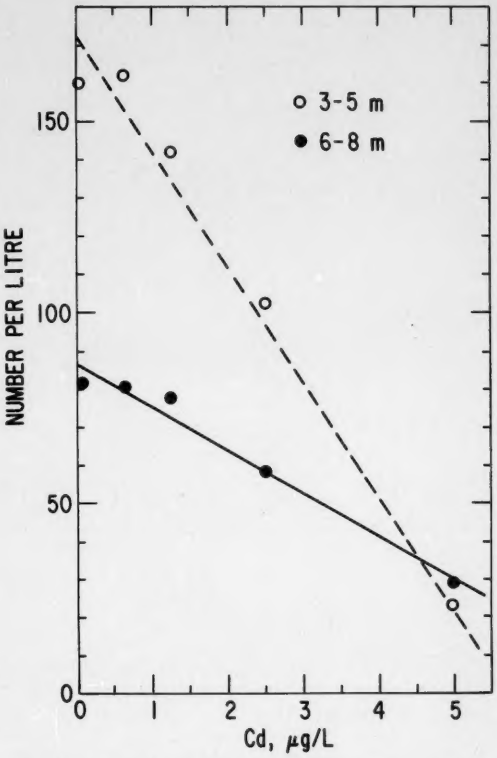

FIG. 5. Effects of cadmium on average numerical density of planktonic crustaceans in Lake Michigan enclosures at different depth (m) ranges. Results shown are for experiment 3.

fidence limits. The linear regression represented in Fig. 6 predicts that *PS* would be reduced by 50% at 5.2 μg Cd/L and 1% at 0.12 μg Cd/L.

DISSOLVED OXYGEN

Measurements of dissolved oxygen in the enclosures used in Lake Michigan indicated effects of cadmium on phytoplankton and the total plankton community. Initial dissolved oxygen concentrations for each experiment were essentially 100% saturation values for the prevailing water temperatures. Average final dissolved oxygen concentrations (mg/L and % saturation) in the enclosures at each cadmium concentration for each experimental period are shown in Table 6. Cadmium effects on plankton community respiration and net photosynthesis were indicated by the relationships between cadmium and final dissolved oxygen concentrations in opaque and translucent (at depths <8 m) enclosures, respectively (Fig. 7). Final dissolved oxygen concentrations were not significantly correlated with cadmium when opaque enclosures (or translucent ones at >9 m) were used. However, when translucent enclosures (<8 m) were used, final dissolved oxygen concentrations were highly (negatively) correlated with cadmium and were significantly reduced by ≧0.2 μg Cd/L. The indicated linear relationship between final dissolved oxygen concentrations in translucent enclosures (% O_2) and cadmium concentration (Cd) is represented by the equation % $O_2 = 95 - 2.4$ Cd. The corresponding correlation coefficient ($r = -0.79$) is highly significant ($P < 0.01$). Thus it appears that

TABLE 4. Hill's numbers (N_0, N_1, and N_2) for a Lake Michigan zooplankton community after in situ incubations with different concentrations of added cadmium during three periods in 1977 (mean ± SE).

Period	μg Cd/L	N_0	N_1	N_2
July 19–Aug. 9	0	10.2±0.05	3.2±0.4	2.0±0.2
	0.6	9.8±0.6	3.4±0.3	2.0±0.1
	1.2	10.0±0.8	4.7±0.7	3.3±0.7
	2.5	8.2±0.5	5.0±0.3	3.8±0.2
	5.0	7.6±0.5	5.0±0.3	4.2±0.2
Aug. 10–30	0	15.1±0.4	7.1±0.5	4.6±0.5
	0.6	14.5±0.4	5.6±0.3	3.9±0.3
	1.2	14.6±0.3	5.8±0.2	4.0±0.2
	2.5	14.2±0.4	6.0±0.4	4.2±0.4
	5.0	12.6±0.5	7.6±0.5	6.0±0.6
Aug. 31–Sept. 21	0	15.5±0.3	8.6±0.6	6.0±0.6
	0.2	15.5±0.3	8.2±0.7	5.6±0.7
	0.4	15.6±0.3	9.1±0.5	6.5±0.6
	0.8	15.6±0.3	9.2±0.4	6.8±0.5
	1.6	14.9±0.2	7.7±0.4	5.6±0.4

TABLE 5. Coefficient of community (*CC*) and percentage similarity (*PS*) for a Lake Michigan zooplankton community after in situ incubations with different concentrations of added cadmium during three periods in 1977 (mean ± SE).

Period	μg Cd/L	*CC*	*PS*
July 19–Aug. 9	0	87.8±2.6	89.5±1.8
	0.6	84.3±3.1	84.1±1.6
	1.2	84.1±3.0	77.4±5.0
	2.5	76.9±2.7	61.8±4.8
	5.0	73.5±3.0	41.4±2.7
Aug. 10–30	0	95.6±1.1	92.6±0.6
	0.6	92.0±1.6	73.7±2.4
	1.2	92.4±1.1	73.3±2.4
	2.5	91.1±1.3	70.4±3.0
	5.0	84.8±2.0	57.8±4.3
Aug. 31–Sept. 21	0	96.9±0.8	90.1±1.3
	0.2	96.1±0.5	81.1±1.2
	0.4	96.5±1.3	78.0±1.9
	0.8	96.5±0.4	74.2±1.7
	1.6	94.0±1.2	70.7±0.8

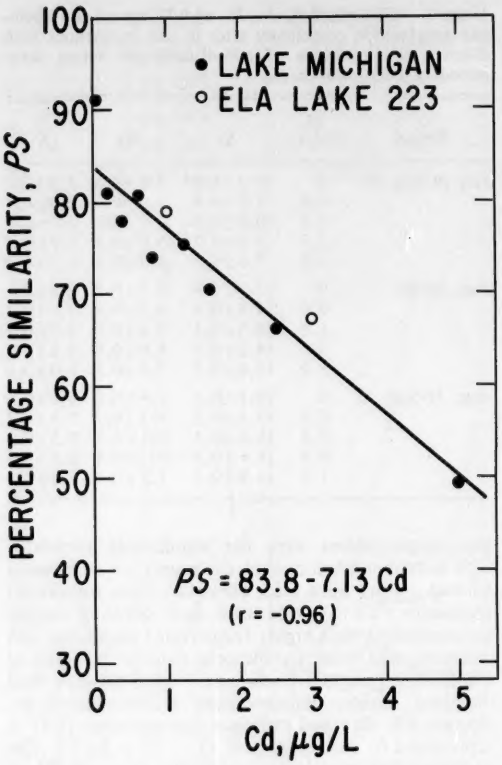

FIG. 6. Effects of cadmium on percentage similarity (*PS*) of planktonic crustacean communities 3 wk after cadmium enrichment of enclosures in Lake Michigan and ELA Lake 223.

plankton respiration was not affected by cadmium, whereas phytoplankton photosynthesis and primary production were significantly reduced.

ENCLOSURE EFFECTS

Enclosure effects on the zooplankton were indicated by the degree of community similarity (*CC* and *PS* values) between samples from the control enclosures and samples from the open lake after 3-wk enclosure periods and by differences in the average densities of specific populations. Average values of *CC* and *PS* for the Lake Michigan enclosures were both significantly different from those for lake samples at the ends of the first two periods, whereas only *PS* was significantly different at the end of the third period (Table 7). The low values of *CC* and *PS* at the end of the first period (August 9) resulted from incubations of opaque enclosures and translucent ones at depths >9 m (too deep). The relatively high values of *CC* and *PS* for the third period coincide with relatively small changes in dissolved oxygen concentrations in the control enclosures during that period (Table 6). The specific proportional abundance differences that contributed most to the reduction of *PS* at the end of the third period (September 21) were higher densities of *Chydorus sphaericus* and lower densities of immature calanoid and cyclopoid copepodites in the enclosures than in the lake (Table 8). Densities of adult *Cyclops vernalis* and *Mesocyclops edax* were also higher in the control enclosures, but average densities of most of the identification categories in these enclosures at the end of the last period were not significantly different from those in the lake (Table 8). Average densities of immature calanoid and cyclopoid copepodites in the control enclosures were significantly lower than those in the lake at the end of each period (Table 8).

Values of *CC* and *PS* for the crustacean zooplankton collected from the control enclosure in ELA Lake 223 on July 21 (20 d after installation) relative to that

TABLE 6. Average concentrations of dissolved oxygen (mg/L and % saturation) in enclosures at each added cadmium concentration after in situ incubations in Lake Michigan during three periods in 1977 (mean ± SE).

Period	Cd μg/L	Dissolved oxygen mg/L	Dissolved oxygen % sat.
July 19–Aug. 9	0	6.6±0.2	74±2
	0.6	6.9±0.2	78±2
	1.2	6.6±0.2	74±2
	2.5	6.9±0.2	78±2
	5.0	6.7±0.2	75±2
Aug. 10–30	0	7.1±0.4	76±4
	0.6	6.5±0.4	69±4
	1.2	6.4±0.4	68±4
	2.5	6.3±0.4	67±4
	5.0	6.0±0.4	64±4
Aug. 31–Sept. 21	0	10.7±0.5	109±5
	0.2	10.0±0.3	102±3
	0.4	9.8±0.4	100±4
	0.8	9.9±0.4	101±4
	1.6	9.7±0.5	99±5

TABLE 7. Average coefficient of community (*CC*) and percentage similarity (*PS*) for Lake Michigan crustacean zooplankton communities in enclosed and unenclosed samples on three dates in 1977 (mean ± SE).

Date	Unenclosed samples *CC*	Unenclosed samples *PS*	Enclosed samples *CC*	Enclosed samples *PS*
Aug. 9	94.5±2.1	93.0±0.3	73.2±1.9	29.0±2.9
Aug. 30	97.0±1.2	91.6±0.2	91.2±1.3	49.4±2.6
Sept. 21	97.0±3.0	91.3±0.1	95.5±0.8	60.9±2.7

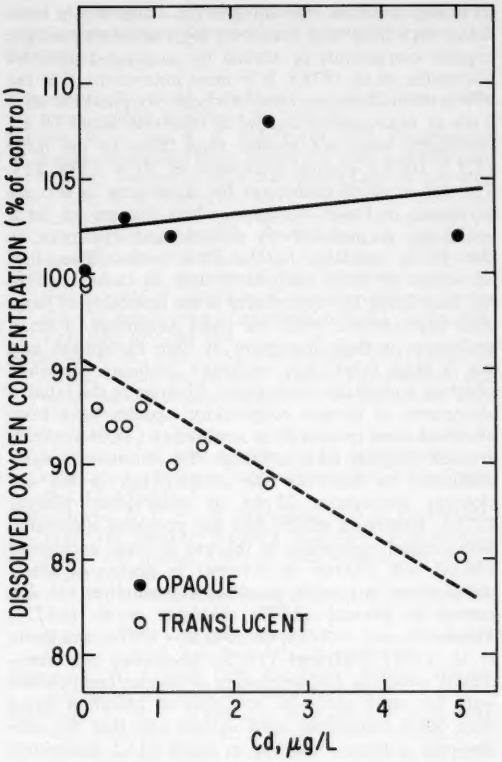

FIG. 7. Effects of cadmium on dissolved oxygen concentration, expressed as percentages of control values, in opaque and translucent enclosures at 3–8 m after 3 wk in Lake Michigan.

from the open lake on July 24 were 59 and 24%, respectively. The values of *CC* and *PS* on August 17 were 67 and 33%, respectively, and on October 28 were 59 and 24%.

Discussion

Four generalizations of importance in assessing the possible long-term effects of increased cadmium concentrations in lakes are: (1) the various indices of stress used in this study differed considerably in their lower limits for detecting cadmium effects; (2) the most sensitive indices (*N'*, *PS*, % O_2) gave no indication of any threshold for cadmium effects at low concentrations; (3) the long-term effects of cadmium would probably be greater than those observed after only a few weeks of exposure; (4) an increase of 0.1 μg Cd/L would probably not have a pronounced effect on the plankton communities in ELA Lake 223 or Lake Michigan. The first conclusion is based on the

TABLE 8. Significance of differences between average density of different categories of Lake Michigan zooplankton in enclosed samples (controls in cadmium enrichment experiments) and unenclosed samples on three dates in 1977, indicating significant differences due to large (+) or smaller (−) populations in the enclosures or insignificant differences (0) at the 5% level.

Category	Aug. 9	Aug. 30	Sept. 21
Holopedium gibberum	−	0	0
Bosmina longirostris	−	0	0
Eubosmina coregoni	−	0	0
Daphnia retrocurva	−	0	0
D. galeata mendotae	0	+	0
D. longiremis	−	−	0
Ceriodaphnia lacustris	0	−	0
Chydorus sphaericus	0	+	+
Cyclops vernalis	0	+	+
C. bicuspidatus thomasi	0	0	0
Mesocyclops edax	0	+	+
Tropocyclops prasinus	−	0	0
Cyclopoid copepodites I–V	−	−	−
Eurytemora affinis	0	+	0
Diaptomus spp.	0	−	0
Calanoid copepodites I–V	−	−	−

observations that the lowest cadmium concentration causing significant effects on *PS*, *N'*, N_0, and % O_2 ranged from 0.2 to 2.5 μg Cd/L. Zooplankton community similarity (*PS*) was relatively sensitive to cadmium in both lakes studied, while species diversity was similarly insensitive. The second conclusion is based on the observations of linear dose–response relationships for *N'*, *PS*, and % O_2 in the Lake Michigan experiments. None of these indices gave any indication of being less sensitive to cadmium in the 0–0.2 μg Cd/L range than in the 0.2–5 μg Cd/L range. The results suggest that even ambient cadmium concentrations may have a small effect on plankton communities. The third conclusion is based on the frequent toxicological observation that toxicity increases with exposure time. Chronic cadmium toxicity to laboratory populations of *Daphnia galeata mendotae*, for example, increased with exposure time up to 12 wk (Marshall 1979) and 22 wk were required to determine long-term average densities (Marshall 1978). The fourth conclusion is based on the linear regressions of *N'* and *PS* on cadmium, both of which predict that an increase of approximately 0.1 μg Cd/L would cause only a 1% effect. The observation of significant effects on *PS* and % O_2 at only 0.2 μg Cd/L, however, suggests that an increase of 0.1 μg Cd/L might have detectable effects. An actual increase of cadmium in lakes would most likely be accompanied by other heavy metals, with which cadmuim has been reported to interact synergistically in their combined effects on phytoplankton abundance and primary production (Gächter 1976; Wissmar 1972). Individually ineffective concentrations of several heavy metals including cadmium were, when

combined, highly toxic to freshwater algae (Wong et al. 1978).

Both direct and indirect (secondary) effects were observed in this study. The direct effects of cadmium included reductions of zooplankton abundance and changes in zooplankton community structure in the experiments in both Lake Michigan and ELA Lake 223, as well as reductions of phytoplankton photosynthesis and primary production (in the Lake Michigan experiments) at much lower concentrations (0.2 μg Cd/L) than previously reported. Indirect effects included increased populations of several species of crustaceans or rotifers at low cadmium concentrations (0–1 μg Cd/L) in both lakes. The interaction of cadmium with light (depth) in their effect on Lake Michigan zooplankton was probably also an indirect effect resulting from the reduction of phytoplankton production (food supply) by cadmium. An apparent stimulation of primary production in the ELA Lake 223 enclosures enriched with $\geqq$10 μg Cd/L was probably an indirect effect due to reduced zooplankton grazing (Schindler et al. 1978). In flow-through chemostat experiments in ELA Lake 223 where zooplankton were excluded, cadmium depressed primary production (F. DeNoyelles, University of Kansas, personal communication). In a recent in situ study of cadmium effects on a marine ecosystem, Kremling et al. (1978) concluded that 1.3 μg Cd/L had no effect on the ecosystem because no marked differences were observed in the phytoplankton. Unfortunately the response of the zooplankton was not measured in that study (K. Kremling, Institut für Meereskunde, personal communication) so there was no evidence to indicate that the lack of apparent effect on the phytoplankton was an indirect effect due to reduced grazing. However, when both phytoplankton and zooplankton responses to copper or mercury enrichment were measured in similar marine experiments, the relative lack of effects on phytoplankton abundance and primary production were attributed to reduced grazing by the zooplankton, which was the component most affected (Beers et al. 1977; Gibson and Grice 1977; Reeve et al. 1976; Thomas et al. 1977a, b).

Our results from the Lake Michigan and ELA Lake 223 experiments are in good agreement. The close agreement for cadmium effects on zooplankton community similarity (*PS*) may be partly coincidental, however, because of decreasing cadmium concentrations in the water in the ELA Lake 223 enclosures. Cadmium disappeared from the water with half-times ranging from 11 to 50 d and by late fall had been quantitatively transferred to the sediments (Schindler et al. 1978). Some of the cadmium added to the Lake Michigan enclosures was also undoubtedly removed from solution by suspended particles. However, a review of the literature (Fleischer et al. 1974) and a study of cadmium sorption (Gardiner 1974) indicate that the fraction removed from solution was probably very small. In the ELA Lake 223 enclosures, almost all of the cadmium remaining in the water was in ionic form; very little was bound by high molecular weight organic compounds or sorbed by suspended particles (Schindler et al. 1978). It is most noteworthy that the effects of enclosure on Lake Michigan zooplankton after 3 wk at near-optimal depths in relatively small (8 L) enclosures were not greater than those in the large (1.5×10^5 L) control enclosure in ELA Lake 223. The use of small enclosures for short-term in situ experiments in Lake Michigan, where the use of large enclosures is prohibitively difficult and expensive, is thus partly validated by the ELA results. The chief advantage of large enclosures such as those used in the ELA Lake 223 experiment is the feasibility of long-term experiments, while the chief advantage of small enclosures is their feasibility of high replication and use in large lakes. Any enclosure probably has some effect on a plankton community. Changes in the relative abundance of certain zooplankton species have been observed even in enormous enclosures (18 000 m^3) in a small English lake, although the dominant species continued to dominate the zooplankton in the enclosures throughout 22 mo of observation (Smyly 1976). Enclosure effects and the problems associated with limited replication in the use of large enclosures (66 m^3 and 1300 m^3 in volume) in studies of heavy metal stress in marine plankton communities are discussed by Menzel (1977), Gamble et al. (1977), Takahashi et al. (1975), Grice et al. (1977), and Beers et al. (1977). Menzel (1977) concluded that community structure and succession of species composition were far more useful as measures of pollution stress than other commonly used indices and that the successional patterns observed in small (4 L) enclosures were similar to those in the large (66 m^3) enclosures.

The present study demonstrates the value of using large and small enclosures jointly in the assessment of pollutant effects in lakes. However, more detailed studies of enclosure effects using different sizes of enclosures simultaneously in different lakes are needed to determine the usefulness of small enclosures. Such studies are presently in progress in Lake Michigan and ELA Lake 382, where a 3-ha bay has been isolated. It is proposed to enrich the bay with cadmium, and we anticipate that a simultaneous experiment with several small enclosures will complement the experiment with the large one. The advantages of each compensate for the disadvantages of the other. In the present study, for example, the close agreement between the two sizes and the observation of significant effects of cadmium additions as low as 0.2 μg Cd/L on Lake Michigan zooplankton in small, highly replicated enclosures leaves little doubt that the changes observed in ELA Lake 223 zooplankton in the single large enclosure enriched with 1 μg Cd/L was due to cadmium stress.

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Experimental Studies on the Effect of Arsenic on Microbial Degradation of Organic Matter and Algal Growth

G. J. BRUNSKILL, B. W. GRAHAM,[1] AND J. W. M. RUDD

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

BRUNSKILL, G. J., B. W. GRAHAM, AND J. W. M. RUDD. 1980. Experimental studies on the effect of arsenic on microbial degradation of organic matter and algal growth. Can. J. Fish. Aquat. Sci. 37: 415–423.

Experiments were performed in tubes in Lake 303 at the Experimental Lakes Area to determine the effects of arsenate and arsenite on microbial degradation of organic matter, and to determine rates of reduction and oxidation of inorganic arsenic. Under winter ice, 40 $\mu mol \cdot L^{-1}$ arsenate or arsenite inhibited microbial degradation of organic matter by 50%. Rates of oxidation of arsenite were about 2 $\mu mol \cdot L^{-1} \cdot d^{-1}$ under aerobic conditions, and rates of reduction of arsenate were about 1 $\mu mol \cdot L^{-1} \cdot d^{-1}$ under anaerobic conditions. During the ice-free season, arsenate and arsenite had little apparent effect upon rates of degradation of organic matter in lake tubes enriched with nutrients. Rates of formation of particulate phosphorus, and rates of planktonic uptake of dissolved phosphorus were depressed in the presence of arsenic. The observed rate of oxidation of arsenite in summer was similar to the winter value. Arsenate reduction rates ($\simeq$30 $\mu mol \cdot L^{-1} \cdot d^{-1}$) were very rapid under short periods of anaerobiosis. In the presence of large nutrient (N, P) concentrations, As did not inhibit the development of high algal biomass.

Based on these experiments, we predict that addition of domestic sewage to arsenate-polluted Kam Lake (near Yellowknife, N.W.T.) will result in a state of restrained eutrophication. Degradation of organic matter will not be inhibited by As in summer, and dissolved phosphorus concentrations will remain high, due to As inhibition of P uptake by the plankton. During the summer, growth of algal blooms may be moderated by As, and more dissolved phosphorus will flow out of the lake to downstream waterbodies.

Key words: arsenic, bacteria, algae, organic matter decomposition, eutrophication

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Des expériences avec tubes ont été effectuées dans le lac 303 de la région des lacs expérimentaux dans le but de déterminer les effets des arséniates et arsénites sur la dégradation microbienne de la matière organique et de déterminer les taux de réduction et d'oxydation de l'arsénic inorganique. Sous la glace en hiver, 40 $\mu mol \cdot L^{-1}$ d'arséniates ou d'arsénites réduisent de 50% la dégradation microbienne de la matière organique. Les taux d'oxydation des arsénites sont d'environ 2 $\mu mol \cdot L^{-1} \cdot jour^{-1}$ dans des conditions aérobies, et les taux de réduction des arséniates sont d'environ 1 $\mu mol \cdot L^{-1} \cdot jour^{-1}$ dans des conditions anaérobies. Durant la saison libre de glace, arséniates et arsénites ont peu d'effet apparent sur les taux de dégradation de la matière organique dans les tubes du lac enrichis d'éléments nutritifs. La présence d'arsénic a pour effet d'abaisser les taux de formation de phosphore particulaire et ceux d'absorption de phosphore dissous par le plancton. Le taux observé d'oxydation des arsénites en été est semblable à celui d'hiver. Les taux de réduction des arséniates ($\simeq$30 $\mu mol \cdot L^{-1} \cdot jour^{-1}$) sont très rapides dans de courtes périodes d'anaérobiose. En présence de fortes concentrations d'éléments nutritifs (N, P), As n'empêche pas la formation d'une grande biomasse algale.

En nous basant sur ces expériences, nous prédisons que le rejet d'égouts domestiques dans le lac Kam (près de Yellowknife, T.N.-O.), déjà pollué aux arséniates, conduira à un état d'eutrophisation restreinte. La dégradation de la matière organique ne sera pas arrêtée par As en été, et les concentrations de phosphore dissous demeureront élevées par suite de l'inhibition par As de l'absorption de P par le plancton. En été, les fleurs algales peuvent être ralenties dans leur croissance par As, et une plus grande quantité de phosphore dissous s'écoulera du lac vers les masses d'eau situées en aval.

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[1]Present address: Hydrology Research Division, Inland Waters, 562 Booth Street, Ottawa, Ont.

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KAM Lake (City of Yellowknife, N.W.T.) currently contains about 40 t of arsenic (30–50 μmol·L^{-1} total dissolved As), which originates largely from atmospheric emissions and tailings pond wastes of a nearby gold mine. Some fraction of the domestic sewage from Yellowknife (population $\simeq$ 8000) has been piped into Kam Lake since 1972, resulting in a large summer phytoplankton biomass (50–80 μg chlorophyll a·L^{-1}) (G. J. Brunskill unpublished data). In 1974, the City of Yellowknife applied for license to dispose of all sewage to Kam Lake, and a review panel was formed to assess the effects of increased sewage additions to the lake, and to assess the utility of Kam Lake as a natural raw sewage treatment facility (Environmental Protection Service 1975). The panel was concerned about the interaction among sewage organic matter, the results of eutrophication, and high arsenic concentrations in the lake. Based on several review papers on arsenic chemistry and toxicity (Ferguson and Gavis 1972; Wallace 1975), the review panel hypothesized that sewage and eutrophication-induced anaerobic conditions in the lake water would allow reduction of arsenate (As^{+5}) to more toxic arsenite (As^{+3}). It was further hypothesized that As^{+5} and As^{+3} would inhibit degradation of sewage organic matter by inhibiting microbial growth (Avron and Jagendorf 1959; Slater 1963; Buttion and Dunker 1971; Jeanjean and Blasco 1970).

This paper gives results of some experiments at the Experimental Lakes Area, Ontario, which attempt to demonstrate the effect of arsenic on the degradation of organic matter, microbial growth, and algal growth.

Materials and Methods

The geography, geology, climate, and physical properties of lakes in the Experimental Lakes Area (ELA) are given in Brunskill and Schindler (1971) and other papers in that special *Journal* issue. This region, near Kenora, Ontario, is hydrologically, and limnologically similar to the Yellowknife region. The arsenic experiments were done at ELA in Lake 303, a shallow ($Z_m = 2.5$ m) small ($A_o = 9.93 \times 10^4$ m^2) lake which normally exhibits moderate O_2 reduction under winter ice (Schindler 1971).

The first set of experiments was done from December 13, 1974 to April 21, 1975 under 20–50 cm of ice. Five 91 cm diameter, 3 m long galvanized steel road culverts were lined with heavy plastic and lowered vertically into the lake sediment from the lake ice. Water depths in these culvert tubes were 1.6–1.8 m. These vertical tubes of lake water were allowed to equilibrate for a few days, and then aqueous solutions of salts of arsenic and of food grade sucrose were added to selected tubes (Table 1). Water samples were taken from these tubes without disturbing O_2 depletion rates under ice cover by pumping water from 8 mm ID tygon tubes inserted at 25 cm depth intervals along the side of the culvert. These tygon sampling tubes were recovered outside the culvert by punching a hole through the ice at a marked site. The volume of water in the vertical culvert tubes in the lake was about 1.15 m^3, but varied somewhat due to ice formation and melting.

These same culvert tubes were used for a second set of experiments in early summer (June 12–July 17, 1975). Lids were made of plywood for the top of the culvert, since the purpose of the experiment was to monitor the effect of As on nonphotosynthetic microbial growth. Experimental additions to the tubes are shown in Table 2. Note that these tubes (No. 1, 2, and 3), and the water therein, are the same as in the first experiment. NaCl was added to estimate tube leakage.

TABLE 1. Experimental treatment of five tubes in Lake 303, ELA, December 13, 1974–April 21, 1975. Average volume of the tubes was 1.15 m^3.

Tube No.	Additions	Day 1, measured concentrations in lake tube
1	none, control	<0.05 μmol·L^{-1} As
2	none, control	<0.05 μmol·L^{-1} As
3	As_2O_3, 6.5 g	42–49 μmol·L^{-1} As^{+3}
4	$Na_2HAsO_4 \cdot 7H_2O$, 20.5 g sucrose 150 g	40–60 μmol·L^{-1} As^{+5}
5	$Na_2HAsO_4 \cdot 7H_2O$, 20.5 g	32–55 μmol·L^{-1} As^{+5}

A third set of experiments was done in Lake 303 from August 18 to October 2, 1975, in 1 m diameter heavy-gauge polyethylene plastic tubes. These tubes were attached to a floating platform at top, and a heavy iron hoop at the bottom, buried in the sediment. Experimental additions to the tubes are given in Table 3.

Arsenic was added as As_2O_3 (As^{+3}) and Na_2HAsO_4 (As^{+5}), N was added as NH_4NO_3, and P as H_3PO_4 (all reagent grade). Food grade sucrose and reagent grade trypticase soy broth (a nutrient and organic matter-rich bacterial medium comparable to domestic sewage) were used as carbon enrichment.

These experimental tubes were sampled at approximately 2-wk intervals. Total dissolved arsenic (TDAs) and phosphorus (TDP), molybdate reactive phosphorus (MRP), arsenate (As^{+5}), and arsenite (As^{+3}) were measured in GF/C filtered water according to Johnson (1971) and Johnson and Pilson (1972) with some modifications. Particulate arsenic (PAs) and phosphorus (PP) were measured from Whatman GF/C filters which were ignited and extracted with dilute acid (Stainton et al. 1974). PAs was also estimated by UV oxidation of filtered and unfiltered samples (with additions of H_2O_2 and H_2SO_4), followed by determinations of TDAs on each sample. Dissolved organic carbon (DOC) and chlorophyll a were measured accord-

TABLE 2. Experimental treatment of three tubes in Lake 303, Experimental Lakes Area, June 12–July 17, 1975. The tubes were covered with a light-tight lid, tube volume 1.3 m^3.

Tube No.	Additions	Expected concentration
1S	none, control (0.2 mol·L^{-1} NaCl)	500 μmol·L^{-1} DOC
2S	223 g Trypticase soy broth (0.2 mol·L^{-1} NaCl)	5000 μmol·L^{-1} DOC N + P high
3S	223 g T.S.B. 6.5 g As_2O_3 (0.2 mol·L^{-1} NaCl)	5000 μmol·L^{-1} DOC $\sim$40 μmol·L^{-1} TDAs

TABLE 3. Experimental treatment of eight tubes in Lake 303, Experimental Lake Area, August 18–October 2, 1975.

Tube No.	Additions	Expected concentration
6	none, control NACl	
7	26.6 g Trypticase soy broth added weekly, NaCl	5000 μmol·L^{-1} DOC N + P low concn.
8	266 g Trypticase soy broth added in one dose, NaCl	5000 μmol·L^{-1} DOC N + P high
9	1.1 g N, 0.074 g P (per week) NaCl	$\sum$N $\sim$ 57 μmol·L^{-1} $\sum$P $\sim$ 2.2 μmol·L^{-1}
10	26.6 g Trypticase soy broth added weekly 63 mmol As^{+5}, NaCl	5000 μmol·L^{-1} DOC 40 μmol·L^{-1} As N + P low
11	270 g Trypticase soy broth added in one dose 63 mmol As^{+5}, NaCl	5000 μmol·L^{-1} DOC 40 μmol·L^{-1} As N + P high
12	1.1 g N, 0.074 g P (per week) 63 mmol As^{+5}, NACl	71 μmol·L^{-1} $\sum$N, 2.2 μmol·L^{-1} $\sum$P
13	63 mmol As^{+5}, NaCl	40 μmol·L^{-1} As N + P low

ing to Stainton et al. (1974). Oxygen in lake waters was measured by the Winkler titration as described in APHA (1965) and Stainton et al. (1974). Adenosine triphosphate (ATP) was measured according to the method of Rudd and Hamilton (1973).

The experimental manipulations were not replicated. Our approach was to add test substances in large doses, so that the responses would be greatly different from the control tubes. The large doses ($\sim$40 μmol·L^{-1} TDAs, 5000 μmol·L^{-1} DOC) were selected to be comparable to known or predicted concentrations in Kam Lake, N.W.T.

Results

Initial laboratory experiments using Kam Lake water indicated that arsenate could be rapidly (<1 d) reduced to arsenite by bubbling with H_2S, but subsequent bubbling with air required 25–35 d to oxidize the arsenite back to arsenate. Since arsenite was thought to be a more toxic form of arsenic, we wanted to determine the rates of reduction and oxidation of arsenic in lake waters subject to deoxygenation and reoxygenation, as would be the case in a eutrophic lake.

TUBES 1 TO 5

The purpose of the first experiment (Table 1), done under 20–60 cm of ice, was to determine the time required for the reduction and oxidation of arsenic. Over the 130 d of the experiment, 20–30% of the arsenic was lost (possibly from tube leakage) from the culvert tubes, and was not found on the plastic tube walls (<0.025%) or in the surface sediments (<0.2% in top 5 cm). The initial additions resulted in a concentration of $\simeq$40 μmol·L^{-1} TDAs, and most of the arsenic remained in the solution phase. Particulate As was always <10% of total arsenic throughout the experiment. Attempts to detect methylarsine gases of As in head-space volumes of sediment samples by gas chromatography gave negative results. Water temperature was 0–2°C, and nutrient concentrations were low (<1 μmol·L^{-1} TDP, <40 μmol·L^{-1} TDN).

The presence of As^{+5} or As^{+3} appeared to inhibit the progress of oxygen depletion (Fig. 1a and b) in the tubes. Oxygen depletion rates per unit area water surface were reduced by 50% in the As spiked tubes, compared to the controls, over the first 90–100 d of the experiment.

At moderate O_2 concentrations (0.02–0.3 mmol·L^{-1} O_2), As^{+3} was oxidized to As^{+5} in 40–50 d (Fig. 2a) at these low water temperatures and in the dark. Since As slowed the rate of O_2 depletion, we added organic carbon (sucrose, see Table 1) to accelerate O_2 depletion in tube 4. On day 17, we bubbled tube 4 with N_2 to hasten the appearance of anaerobic conditions (Fig. 2b). After the onset of anaerobic conditions, As^{+5} was reduced to As^{+3} in about 40 d (Fig. 2b).

TABLE 4. The effect of the ratio TDAs:TDP on algal biomass and chlorophyll in tubes 10–13 (see Table 3), August 18 to October 2, 1975.

Tube No.	Initial TDAs (μmol·L^{-1})	Initial TDP (μmol·L^{-1})	Initial TDAs/TDP	Final biomass (mg·L^{-1})	Final chlorophyll (μg·L^{-1})
10 (Pulsed addition of trypticase soy + As)	41	8.1	5	40.8	304
11 (Trypticase soy in one dose, As)	42	98	0.4	10 000	1110
12 (Inorganic N & P added weekly, As)	37	1	37	39.1	93
13 (As^{+5} only)	41	0.5	82	12.3	24

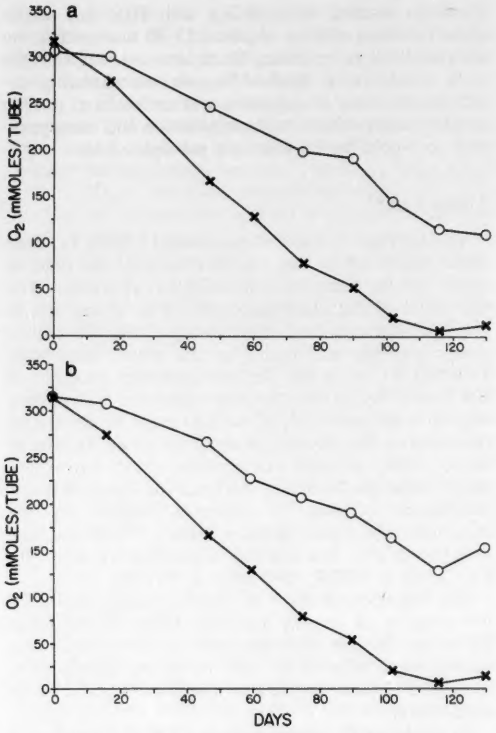

FIG. 1. Variation in the mass of dissolved oxygen (O_2 in mmol per tube) against time in days (December 13, 1974 to April 21, 1975). a) Tube 3 (○) received an addition of As^{+3} (6.5 g of As_2O_3) at the beginning of the experiment, whereas control tube 2 (×) received no arsenic addition. b) Tube 5 (○) received an addition of As^{+5} (20.5 g of $Na_2HAsO_4 \cdot 7H_2O$) at the beginning of the experiment, whereas control tube 2 (×) received no arsenic additions.

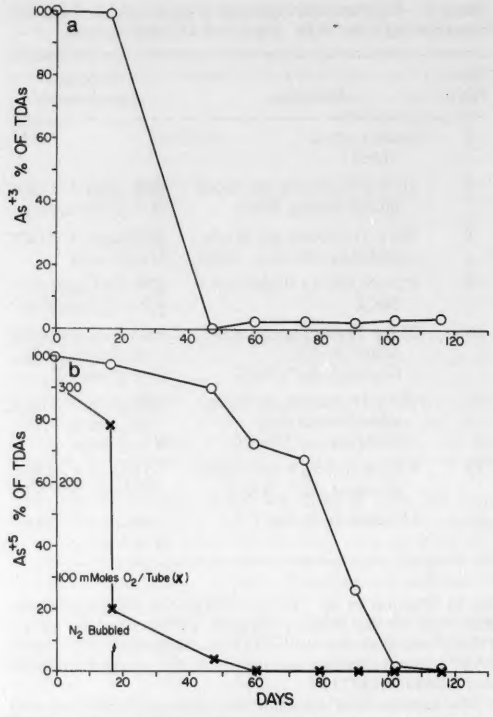

FIG. 2. The percentage of total dissolved arsenic (TDAs) in tube 3 against time in days (December 13, 1974 to April 21, 1975). a) Tube 3 received an addition of As^{+3} (6.5 g As_2O_3) at the beginning of the experiment. The disappearance of As^{+3} was matched by an equivalent increase in measured concentrations of As^{+5}, indicating essentially complete oxidation of As^{+3} to As^{+5}. b) Tube 4 received an addition of As^{+5} (20.5 g of $Na_2HAsO_4 \cdot 7\ H_2O$) at the beginning of the experiment. On day 17 of the experiment, tube 4 was bubbled with N_2 to remove O_2 from the water column. O_2 concentrations (×) in this tube were undetectable on day 60, and As^{+5} decreased to a negligible proportion of TDAs shortly thereafter. The disappearance of As^{+5} was matched by an equivalent increase in As^{+3}, indicating an essentially complete reduction of A^{+5} to As^{+3}.

These winter experiments indicated that arsenic reduction and oxidation between the +5 and +3 states was sluggish in these lake waters at 0–2°C and in the dark. Microbial activity was likely very low under these conditions. The experiments also indicated that arsenic (+5 or +3) inhibited O_2 depletion, which is a result of microbial degradation of organic matter. Our next experiment was designed to test this observation at warmer water temperatures, and with an extra supply of nutrients.

TUBES 1S TO 3S

Light-excluding lids (not air-tight) were placed on three of the same plastic-lined culvert tubes used in the first experiment, to provide a lake environment for nonphotosynthetic growth. Water temperature was between 17 and 25°C throughout the experiment (June 12–July 17), and there was no thermal stratification in the lake or in the tubes. Loss of solutes from tube leakage was estimated from measurements of Na and Cl, added as a tracer. There was 0–10% loss of arsenic from the tube water columns in this experiment. The tube lid and exclusion of light from the control tube water column apparently caused a 30% reduction in O_2 concentration, compared to nearby open-topped, clear plastic tubes used in a following experiment. The addition of large amounts of dissolved organic matter and nutrients (trypticase soy broth, Table 2) caused

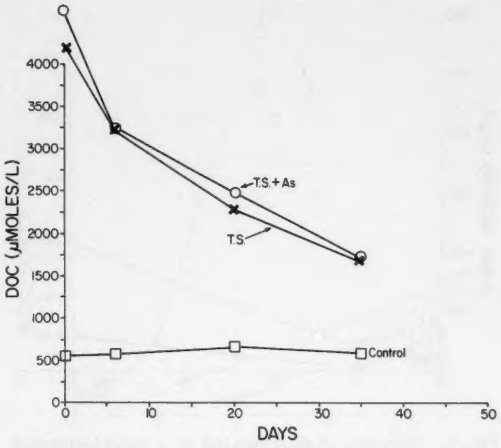

FIG. 3. Variation in dissolved organic carbon (DOC) in control (□), tube spiked with trypticase soy broth (×), and tube spiked with trypticase soy broth plus As^{+5} (○) in Lake 303 experimental tubes, June 12 to July 17, 1975.

complete O_2 depletion in less than 6 d in tubes 2 and 3. Initial nutrient concentrations were very high (TDN = 800–1000 $\mu mol \cdot L^{-1}$, TDP = 80–100 $\mu mol \cdot L^{-1}$) in these tubes and declined slowly to TDN = 700 $\mu mol \cdot L^{-1}$ and TDP = 10 $\mu mol \cdot L^{-1}$ by the end of the experiment.

The presence of As did not greatly affect the rate of degradation of the added DOC (Fig. 3). Minimum rates of degradation of DOC for the aerobic part of the curve (day 0 to 6 in Fig. 3) were 175 to 200 $\mu mol \cdot L^{-1}$ DOC $\cdot d^{-1}$ for tubes 2S and 3S, whereas under anaerobic conditions the microbiota metabolized ≃50 $\mu mol \cdot L^{-1}$ DOC $\cdot d^{-1}$ in the presence or absence of As. Arsenic was added to tube 3S as As^{+5} (see Table 2): after day 6 of the experiment, all arsenic was found to be As^{+3} at concentrations of 30–40 $\mu mol \cdot L^{-1}$ under anaerobic conditions.

The presence of As inhibited the uptake of the dissolved phosphorus from the trypticase soy broth into the suspended particulate phase (largely microbial biomass) (Fig. 4a and b). Rates of TDP disappearance from the tube water were similar in the presence and absence of As (6–8 $\mu mol \cdot L^{-1}$ TDP $\cdot d^{-1}$) for the aerobic, As^{+5}, initial phase of the experiment (day 0–6). During the anaerobic, As^{+3} phase of the experiment (day 6–35), the presence of arsenic inhibited the disappearance (= uptake) of TDP by 50%, compared to control tube 2S rates of 1.5 $\mu mol \cdot L^{-1}$ TDP $\cdot d^{-1}$ (Fig. 4b).

Measurements of ATP on seston from the tubes indicated that As had little or no effect on microbial biomass ATP. This experiment thus indicated that As does not have a significant effect on microbial degradation of organic matter in the presence of excess nutrients

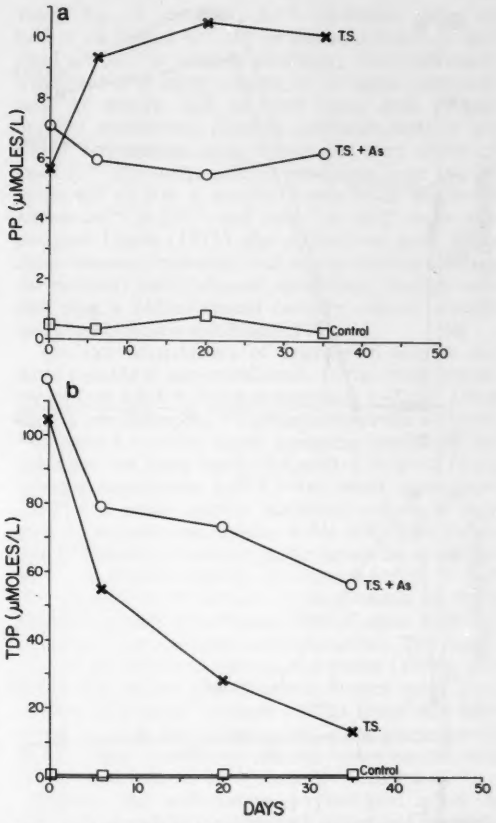

FIG. 4. Variation in a) particulate phosphorus (PP), and b) total dissolved phosphorus (TDP) in Lake 303 experimental tubes spiked with trypticase soy broth (×), and trypticase soy broth plus As^{+5} (○), June 12 to July 17, 1975.

and at warm water temperatures. It did, however, decrease the rate of P uptake by heterotrophs.

TUBES 6 TO 13

A third experiment was designed to demonstrate the effect of As on the phytoplankton and microbial community. In open-top plastic tubes (Table 3), <10% of added As was lost by leakage. With addition of trypticase soy broth, tubes 8 and 11 were quickly depleted of O_2, and the added As^{+5} was completely reduced to As^{+3} in 2 d (Fig. 5a and b). After partial degradation of the trypticase soy broth and liberation of inorganic nutrients (day 24), O_2 concentrations were very high, probably due to rapid phytoplankton growth, and As^{+3} was completely oxidized to As^{+5}. Due to infrequent sampling, rates of these reactions cannot be estimated. Deoxygenation and reoxygenation rates in the tube

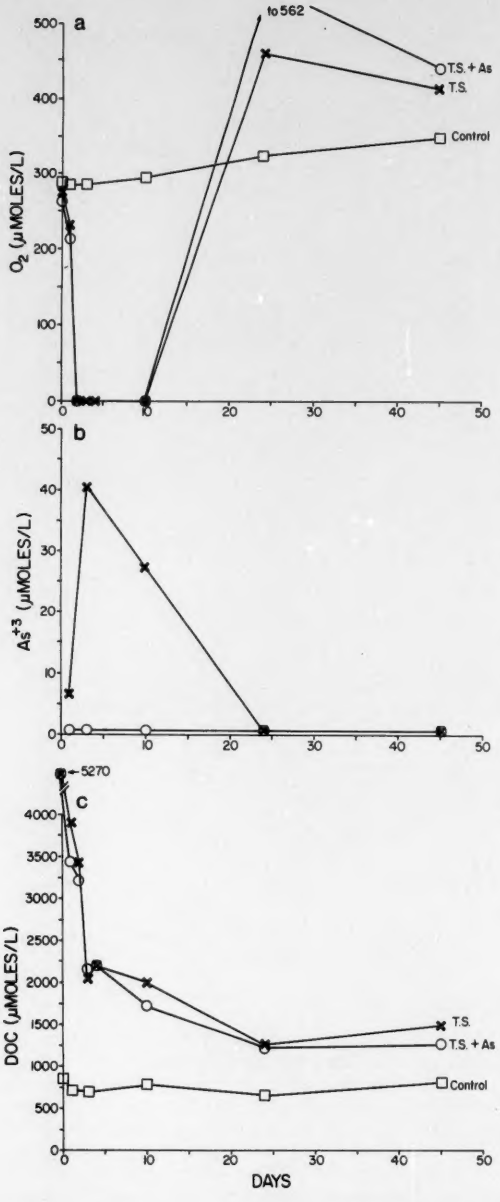

FIG. 5. Variation in a) oxygen, b) As^{+5}, and c) dissolved organic carbon (DOC) in experimental tubes spiked with trypticase soy broth (×), and trypticase soy broth plus As^{+5} (○), August 18 to October 2, 1975.

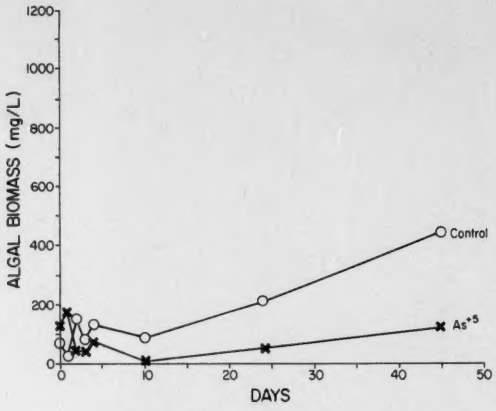

FIG. 6. Variation of algal biomass in a control tube and a tube with As^{+5} only added, in Lake 303, ELA, August 18 to October 2, 1975.

water column did not appear to be affected by As (Fig. 5a and b). The decrease of DOC with time (due to microbial degradation) in the trypticase soy broth tubes, with and without As, was very similar (Fig. 5c).

Arsenic alone (tube 13) had little effect on variations in concentrations of PP, TDP, or chlorophyll *a* throughout this experiment, but PN, PC, and agal biomass were depressed by 50% in the presence of As^{+5} (for example, see Fig. 6), compared to the control tube. In the tubes enriched with inorganic N and P only, the presence of As appeared to have little effect on algal biomass, and delayed the increase in chlorophyll *a* with time. PC, PN, and PP were slightly depressed in the presence of As in this experiment. In the tubes enriched with trypticase soy broth, however, PC, PN, PP, chlorophyll *a* and algal biomass were depressed 50% or more in the presence of As (Fig. 7a and b).

Discussion

Many studies have shown that arsenic is found primarily in the sediment phase of aquatic ecosystems (Onishi and Sandell 1955; Boström and Valdes 1969; Seydel 1972; Huang 1975). Barrow (1974) showed that arsenate displaces phosphate from reactive sites on soil particles, and Wauchope (1975) found that arsenate (and methylarsenates) was more strongly sorbed by soil slurries than was phosphate. In soils, Wauchope (1975) and Woolson et al. (1973) found that As was preferentially bound to clay fractions rich in Fe, Al, and Mn; Kanamori and Sugawara (1965) and Crecelius (1975) report similar associations in lake sediments.

For the duration of our short-term experiments (1–2 mo), we found that our high concentrations of As be-

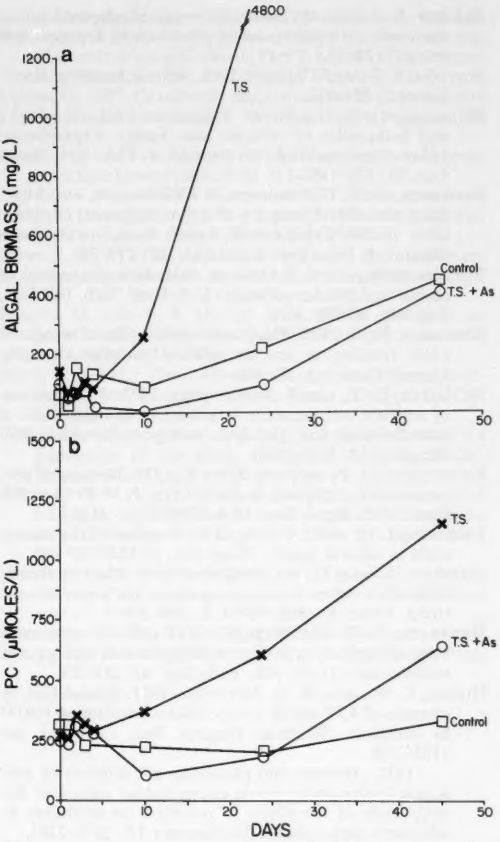

FIG. 7. Variation of a) algal biomass and b) particulate carbon (PC) in Lake 303 experimental tubes spiked with trypticase soy broth (×) and trypticase soy broth plus As^{+5} (○), August 18 to October 2, 1975.

haved rather conservatively. Most (95–100%) of the added arsenic remained in the dissolved phase, and little loss of As from the tubes was noted, after correction for tube leakage. Little or no As was detected in surface sediments (see Brunskill et al. 1971 for major element sediment chemistry), in the plastic tube walls, or in suspended particulate matter. When phosphorus was added with As to some tubes, P was rapidly taken into the particulate phase and transported to the sediments, whereas As remained in the solution phase.

Johnson and Pilson (1975) derived an equation that predicts the rate of arsenite oxidation in seawater, based on laboratory experiments in which temperature, As^{+3} concentration, pH, and salinity were varied. Extrapolated to the conditions of our freshwater lake tubes, their Eq. 5 predicts As^{+3} oxidation rates of 0.03×10^{-3} $\mu mol \cdot L^{-1} \cdot d^{-1}$ in winter, and 0.21×10^{-3} $\mu mol \cdot L^{-1} \cdot d^{-1}$ in summer conditions. Our experiments were done with about a factor of 10 higher concentrations of arsenic, and, in some cases, with extreme nutrient enrichment. Arsenite oxidation rates in our winter and summer tube experiments were about 1.9 $\mu mol \cdot L^{-1} \cdot d^{-1}$ under aerobic conditions, over the pH range 6.5 to 8.0, a specific conductance of 20-180 $\mu mhos \cdot cm^{-1}$ at 25°C, and from 1 to 25°C water temperature. Lunde (1977) also pointed out great differences between freshwater and marine environments in As chemistry and biological metabolism. Sunlight may also play a photochemical catalytic role in arsenite oxidation (Johnson and Pilson 1975).

The rate of reduction of arsenate to arsenite was more variable in our experiments. In the winter experiments (pH 6.5–6.9, water temperature 1–2°C), under aerobic conditions, As^{+5} was apparently reduced slowly (0.1 $\mu mol \cdot L^{-1} \cdot d^{-1}$); under anaerobic conditions, the reduction was more rapid (1.3 $\mu mol \cdot L^{-1} \cdot d^{-1}$). In the summer experiments (pH 6.9–8.0, water temperature 20–25°C), under aerobic conditions and rapid algal growth, arsenate was usually stable with little indication of reduction; however, under anaerobic conditions As^{+5} was reduced rapidly ($\simeq 30$ $\mu mol \cdot L^{-1} \cdot d^{-1}$) (see Fig. 2a and b, 5a and b) in the presence of rapid bacterial growth (D. Planas, 6690 Hudson Rd., #3, Montreal, Que., personal communication). This is contrary to the results of Johnson and Burke (1978), who found that marine phytoplankton blooms cause a reduction of arsenate. Johnson (1972) found that laboratory seawater bacterial cultures in log phase growth could reduce arsenate to arsenite under aerobic conditions at a rate of 10^{-11} $\mu mol \cdot L^{-1} \cdot cell^{-1} \cdot min^{-1}$.

Despite the well known physiological effect of arsenic in the inhibition of mitochondrial and glycolytic energy metabolism, and in reducing the cellular concentrations of ATP in bacteria (Ordal and Goldman 1975; DeMaster and Mitchell 1973; Huang and Mitchell 1971 and 1972), As^{+5} and As^{+3} appeared to have little effect on microbial degradation of organic matter in our nutrient-rich warmwater experiments (Fig. 3 and 5c). With no nutrient or organic matter enrichment, and under winter ice, microbial activity (as estimated by rates of O_2 depletion) was reduced by 50% in the presence of As^{+5} or As^{+3} (Fig. 1a and b). Whether this effect was due to temperature, lack of nutrients, or both, cannot be decided from these data.

Our data do show that phosphorus uptake by plankton is slowed by As^{+5} or As^{+3} (Fig. 4a and b). Planas and Healey (1978) also found that arsenate inhibited P uptake in laboratory cultures of *Chlamydomonas reinhardi*, and, as in our work, little As was taken up by the organisms. We made a few measurements of ATP on filters from the lidded tubes of the June 12–July 17, 1975 experiment, and found that ATP concentrations were not affected by the presence of As^{+5}.

In contrast, G. J. Brunskill (unpublished data) found that spring and summer phytoplankton blooms in Kam Lake, N.W.T., incorporated about 50% of total arsenic into their biomass, and that the resulting 30 μmol·L^{-1} PAs was regenerated into the TDAs pool in the month following the plankton bloom. Irgolic et al. (1977) measured uptake and metabolism of As in an alga and *Daphnia magna*, and found an organoarsenic compound in the lipid fraction. D. Planas (personal communication) has studied phytoplankton samples of our tube experiments, and found that there were no great changes in species composition as a result of As addition (Planas and Healey 1978). In most of our experiments, As deleteriously affected some parameters of algal growth in one way or another. Not all treatment pairs of tubes (with and without As) allowed consistent interpretations: in one case the addition of As to a highly nutrient enriched tube resulted in increased algal growth. Table 4 indicates that at high As:P ratios, algal biomass and chlorophyll were depressed, compared to low As:P tube data. Giddings and Eddlemon (1978) also demonstrated the effect of As on photosynthesis and respiration in aquatic communities.

The effect of the 40 μmol TDAs·L^{-1} on sewage degradation in the eutrophic waters of Kam Lake is probably small, because nutrient concentrations are high. More of the sewage-supplied P will remain in the dissolved phase for a longer period of time and will be available to the outflow. Algal growth will likely be depressed by arsenic even in the presence of excess nutrients.

From our present experimental work, it would appear that As is not rapidly incorporated into the food chain through plankton. Numerous authors (Penrose 1974; Reay 1972; Wagemann et al. 1978) have tabulated arsenic concentrations in aquatic organisms, and from these papers it appears that As is not biologically accumulated by passing upwards through the food chain. Toxicity of arsenic is not well established, partly because the element occurs in various forms (Penrose 1974; Schaefer and Pipes 1973; Surber and Meehean 1931; Gilderhus 1966; Rocovich and West 1975; Wildenberg 1978).

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Continuous Culturing of Natural Phytoplankton Communities in the Experimental Lakes Area: Effects of Enclosure, in situ Incubation, Light, Phosphorus, and Cadmium

F. deNOYELLES JR.

Division of Biological Sciences, University of Kansas, Lawrence, KS 66045, USA

R. KNOECHEL

Department of Zoology, Erindale College, University of Toronto, Mississauga, Ont. L5L 1C6

D. REINKE, D. TREANOR, AND C. ALTENHOFEN

Division of Biological Sciences, University of Kansas, Lawrence, KS 66045, USA

deNOYELLES, F., JR., R. KNOECHEL, D. REINKE, D. TREANOR, AND C. ALTENHOFEN. 1980. Continuous culturing of natural phytoplankton communities in the Experimental Lakes Area: effects of enclosure, in situ incubation, light, phosphorus, and cadmium. Can. J. Fish. Aquat. Sci. 37: 424–433.

Continuous culture chambers containing and receiving raw lake water as an inflow were incubated in the laboratory and in situ. This was done to determine the suitability of continuous algal culture for recording the responses of lake phytoplankton to experimentally imposed perturbations simulating environmental changes. The distribution of the phytoplankton in control chambers, particularly with in situ incubation, remained similar to that of the lake. Other chambers produced changes in distribution as responses to perturbations of filtered inflow, light, a nutrient, and cadmium. These responses were consistent with existing data for lakes at the Experimental Lakes Area, including the responses to cadmium which were compared to those from tube experiments. The relative merits of these and other types of enclosures including batch cultures, large bags, and tubes are discussed.

Key words: phytoplankton culture, enclosure effects, phosphorus, light, cadmium, bioassay, microcosms

deNOYELLES, F., JR., R. KNOECHEL, D. REINKE, D. TREANOR, AND C. ALTENHOFEN. 1980. Continuous culturing of natural phytoplankton communities in the Experimental Lakes Area: effects of enclosure, in situ incubation, light, phosphorus, and cadmium. Can. J. Fish. Aquat. Sci. 37: 424–433.

Des chambres de culture continue contenant et recevant, comme entrée, de l'eau de lac non traitée ont été incubées en laboratoire et in situ. Cela dans le but de déterminer si une culture continue d'algues peut servir à enregistrer les réponses du phytoplancton lacustre à des perturbations produites expérimentalement et simulant des changements dans l'environnement. La distribution du phytoplancton dans les chambres témoins, en particulier avec incubation in situ, demeure semblable à celle du lac. Les autres chambres accusent des changements de distribution en réponse à des perturbations telles que entrée d'eau filtrée, lumière, un élément nutritif et cadmium. Ces réponses s'accordent avec les données déjà recueillies dans les lacs de la région des lacs expérimentaux, y compris réponses au cadmium, que nous avons comparées avec celles observées au cours d'expériences avec tubes. Nous examinons les mérites relatifs d'enceintes de ce type, ainsi que d'autres, y compris cultures en grande quantité, grands sacs et tubes.

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APPLYING a perturbation to a lake provides the most direct experiment for studying a lake's response to a disturbance. Another approach has involved the use of various types of microcosms enclosing portions of the lake. By containing the perturbation, the surrounding habitat is protected while the enclosed portion develops a response which may be extrapolated to the whole lake. Phytoplankton are amenable to study in enclosures because of their size and life histories. Though many types of enclosures have been used for work with phytoplankton (Strickland and Terhune 1961; Goldman 1962; Antia et al. 1963; Málek et al. 1969; Soeder and Talling 1969; Barlow et al. 1973; deNoyelles and O'Brien 1974; Schindler et al. 1974; Gerhart and Likens 1975; Blinn et al. 1977; Venrick et al. 1977),

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we will demonstrate the advantages of continuous culturing performed in the laboratory and in situ.

Continuous culturing has long been recognized as the best laboratory method for growing a wide variety of aquatic microorganisms. This is largely a function of the continuous renewal of the culture medium (Málek et al. 1969). Most often an enclosure is operated as a chemostat with a culture of a single species attaining growth at equilibrium with a chemical input (Málek et al. 1969). As it is our intention to extrapolate culture responses to the lake phytoplankton community, we enclosed a sample of the community rather than considering any single species to be a representative "test organism."

A few investigators have used samples of phytoplankton communities in laboratory continuous cultures (Barlow et al. 1973; Gerhart and Likens 1975; O'Brien and deNoyelles 1976). However, their method used filtered lake water as the inflow without the outflow also being filtered and this may subject the enclosed community to a dilution pressure reducing its similarity to the lake community. Our improvement using unfiltered lake water for the inflow allows the continuous renewal of the cultures with phytoplankton from the lake community and maintains their similarity in the absence of perturbation. Perturbed cultures are continually renewed through time by the inflow. Organisms which have already responded leave and the new ones must develop the same response if the observed culture response is to continue. All types of enclosures necessarily subject the contained organisms to some unnatural conditions. The renewal also reduces some of these conditions by reducing the residence time of the organisms.

A possible disadvantage of using unfiltered inflow concerns the response to perturbation which is diluted by the inflow of the lake community. This affects the detection limit for the response, but we can compensate by using more sensitive analytical procedures. Thus a reduced detection limit due to inflow for observing a change in species distribution can be compensated for by increasing the cell count, as will be discussed later.

In the present study, laboratory and in situ continuous cultures were used to observe changes in species distributions of phytoplankters to filtered inflow, nutrient, light, and toxicant perturbations. An evaluation of this method includes the measurement of enclosure effects. By demonstrating successful maintenance of the natural species composition in the absence of perturbation, altered distribution in response to perturbation may be more accurately extrapolated to the lake community.

Materials and Methods

Continuous Culturing Systems

The laboratory continuous culturing system consists of four culture flasks (5 L, glass jacket, round bottom, distilling style) positioned around a central reservoir (20 L, glass bottle, carboy style). Each culture flask is drained by a peristaltic pump which in turn draws the inflow from the reservoir. Liquid perturbations are introduced by another pump, and mixing is maintained by a magnetic stirring bar. Each culture flask is encircled by five fluorescent lamps (32 W, cool white) which together supply 150 $\mu Ei \cdot m^{-2} \cdot s^{-1}$ to the surface of the flask. Such an intensity, though of a different quality, on the average would be found between 2 and 3 m in the lakes studied. Culture temperature is regulated by an external circulating water bath fitted with refrigeration coils and set at the prevailing lake condition at the depth of the in situ incubation. Water for the laboratory cultures was collected adjacent to the in situ culture inlet.

The in situ continuous culturing system (Fig. 1) was that described for pond use (deNoyelles and O'Brien 1974) with some alterations of the suspension and sampling systems for lake incubation. Each culture flask (5 L, round bottom, distilling style) is connected by a tube to its own collection bottle (13 L, glass bottle, carboy style), which is in turn connected to a surface air valve (Fig. 1). The culture flask is initially rapidly filled from the lake through an inlet tube (3.2 mm ID), with air escaping directly to the surface and with the tube to the collection bottle closed. Once full, the direct connection of the culture flask to the surface is clamped off and the valve regulating the escape of air from the collection bottle is opened. With the tube to the collection bottle opened, hydrostatic pressure forces water through the culture flask at a rate equal to the escape of air from the collection bottle. Liquid perturbations are gravity fed or periodically injected. Mixing is maintained by a magnetic stirring bar driven by a D.C. motor with speed adjusted from the surface. Both in situ and laboratory culturing systems were operated with inflow at 50 or 60 $mL \cdot h^{-1}$, which for a 5.5-L culture volume gave a retention time of 110.0 or 91.7 h. All system components in contact with organisms are of pyrex, silicon or teflon, and are acid-washed and autoclaved prior to each incubation.

Experiments

Experiment 1 was conducted during July 1976 in the epilimnion of L239 at a depth of 90 cm. It was designed to evaluate the physical operation of both culture systems and the effects of enclosure on the phytoplankton using four laboratory and two in situ cultures. Two of the laboratory cultures and both in situ cultures received (60 $mL \cdot h^{-1}$) filtered (Gelman type A glass fiber filters) lake water. The phytoplankton species composition was monitored during the first 144 h and the remaining 236 h was used to assess physical operation of the systems. Only one in situ culture was analyzed for phytoplankton responses while the other was used to assess the physical operation of the system. The laboratory cultures were supplied with 150 $\mu Ei \cdot m^{-2} \cdot s^{-1}$ of illumination at the culture flask surface for a 13 h daily exposure. A similar average intensity reached the in situ cultures with the partial shading they received from the float.

Experiment 2 was conducted during August 1976 in the upper hypolimnion of the SW basin of L226 at a depth of 6.5 m. It was designed to study the effects of enclosure and the responses to increased phosphorus and light for a hypolimnetic algal community. Also, the effect of the greater depth on the flow in the in situ system was closely

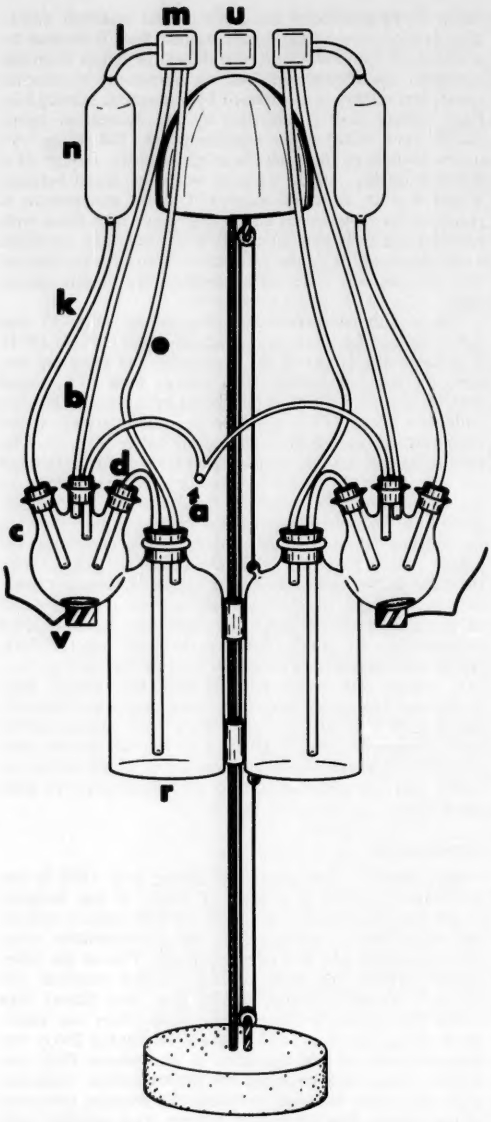

FIG. 1. Diagram of an in situ continuous culturing system with two culture chambers (*c*) each connected by *b* directly to the surface and by *d* to a collection bottle (*r*) which is in turn connected by *e* to an air valve at *m*. Each culture receives lake water inflow through *a* with regulation at *m* and liquid perturbation inflow through *k* from *n* with regulation at *m* through *l*. Magnetic stirrers (*v*) regulated at *u* provide culture mixing. Cultures are positioned along a cable which is attached to an anchor and float.

followed. The four laboratory and two in situ cultures received (60 $mL \cdot ^{-1}$) unfiltered lake water for 267 h. One of the laboratory cultures received a P increase and another received increased light. For the laboratory incubation to approximate physical conditions at 6.5 m in the lake, the laboratory cultures were maintained at 10°C and three cultures received 15 $\mu Ei \cdot m^{-2} \cdot s^{-1}$ of light (surface of culture). The high light culture received 30 $\mu Ei \cdot m^{-2} \cdot s^{-1}$. The photoperiod was 13 h for all laboratory cultures. The high P culture received an immediate input of 10 $\mu g\ P \cdot L^{-1}$ (H_3PO_4) followed by a continuous inflow of 0.2 $\mu g\ P \cdot L^{-1} \cdot h^{-1}$ (H_3PO_4), and after several days the theoretical concentration of 20 $\mu g\ P \cdot L^{-1}$ was approached. Lake water for the laboratory cultures was drawn from the lake next to the inlet of the in situ cultures. Since phytoplankton biomass in the hypolimnion changed rapidly with depth, it was particularly important to draw water from the same vertical position for in situ inflow, laboratory inflow and lake samples to maintain continuity between the three. It was also necessary to keep the lake water at low temperature and in dim light during collection and transportation. In the laboratory the inflow water was incubated at the same temperature and dim light conditions used for the cultures and was replaced every 48 h.

Experiment 3 was conducted during August 1977 in the epilimnion of L239 at a depth of 174 cm using four in situ cultures. It was designed to study the effects of enclosure and the responses to cadmium of a phytoplankton community. Comparisons were also to be made with a tube experiment. Two cultures served as controls while two others received Cd injections every 24 h for 188 h of incubation with inflow of unfiltered lake water at 50 $mL \cdot h^{-1}$. Considering the dilution rate, and with periodic instead of continual Cd addition, Cd concentration beginning with the first injection should have remained between 3 and 4 $\mu g \cdot L^{-1}$. Actual concentrations were determined by two methods: by measuring Cd^{115} (Unilux II liquid scintillation spectrometer) which was combined with the injected Cd, and by measuring total Cd with atomic absorption (Varian Techtron type AA-5 with a CRA 90 carbon rod atomizer). The measured concentrations of Cd in unfiltered samples determined on six occasions remained between 2.9 and 4.2 $\mu g \cdot L^{-1}$. From 1 to 5% of this total was present in the particulate fraction of the sample. Following 208 h of incubation with inflow (20 h before Cd injection plus 188 h with Cd), incubation without flow or Cd injection continued for another 94 h.

The tube study which provided a comparison with the cultures was begun a month earlier as part of a study of the effects of cadmium in L223 (Schindler et al. 1979). The tubes, constructed of nylon-reinforced polyethylene, were 10 m in diameter and extended from the surface to the bottom at a depth of 2 m. The tube wall was sealed into the sediment by piling a continuous ring of rocks around the edge. Five tubes were placed in the lake a few days prior to the start of the experiment. One tube was used for each of 4 Cd levels (1, 3, 10 and 30 $\mu g\ Cd \cdot L^{-1}$) and the last was reserved as a control. Cadmium was added to the tubes only at the beginning of the experiment. By day 14, which was the interval used for the comparison with the in situ continuous cultures, the Cd concentrations had declined in each to about half of the original.

PHYTOPLANTON ANALYSES

Phytoplankton samples (100 mL) were collected through

tubes from the cultures and from the lake through a tube attached next to the inlet of the in situ cultures. The samples were preserved in acid Lugol's, concentrated in settling chambers, and counted with a Wild inverted microscope. Counting detection limits for individual species ranged between 25 and 300 organisms per litre according to their size (i.e. magnification used), with the lower detection limits for the larger organisms. Organisms used in the analyses with less than a 20 μm diameter were generally more abundant than $20\,000 \cdot L^{-1}$, while larger ones ranged from 100 to $2000 \cdot L^{-1}$.

Culture and lake communities were compared at the beginning and end of the first two experiments through similarity indices which used 21–25 of the more abundant species. Species numbers from microscopic counts were first converted to biomass using 50 cell volume measurements for each species. The indices include % similarity as $\%S = \min(P_{ia} \text{ or } P_{ib})$, Stander's index (equation 1) (Sullivan 1975) and euclidean distance (equation 2)

$$(1)\quad \text{SIMI} = \frac{\sum_{i=1}^{s} P_{ia} P_{ib}}{\sqrt{\sum_{i=1}^{s} P_{ia}^2}\sqrt{\sum_{i=1}^{s} P_{ib}^2}}$$

$$(2)\quad \text{Cb} = \sum_{i=1}^{s} (P_{ia} - P_{ib})^2$$

where: P_{ia} and P_{ib} are biomass values of species i in samples a and b. Two euclidean distance values were generated based on different biomass representations, one with the species absolute biomass (Cb) and the other with the species biomass as a percent of the total biomass of the species used (Cb%). For both values, the mean standard deviation option in BMDP-2M was used to standardize the data (Dixon 1975). Euclidean distance values theoretically vary from 0 for identical communities to infinity with increasing dissimilarity while the other indices vary from 0 to 100% similarity. In experiment 3 four selected species also common in the tube study were counted and their distributions throughout the incubation period were compared.

For experiments 1 and 2 tests were used to estimate the level of significant difference between similarity values when comparing community samples. In performing the *T*-tests, the individual similarity values were used, though only means are given in Tables 1 and 2. It was also necessary to first change each %S and SIMI value using arcsin transformation to approximate normal distribution for the original percent values. For experiments 2 and 3 where the distribution of particular species was considered, significant differences between control cultures and treatment cultures for a given species on a given day were detected by subjecting paired 100-field counts from each culture to a nested analysis of variance (p. 268, Sokal and Rohlf 1969) with a priori test of means of treatment vs. control and lake vs. control.

Response Detection

One minor drawback of the continuous culture approach is dilution of the species response as compared to a closed system (batch culture). The summation of all growth and loss processes can be represented by the net growth constant k, calculated as $(\ln (n_t/n_o))/t$ for a batch culture. In continuous culture the observed net growth constant k' is reduced by the proportion $Q/(Q + V)$ where Q is the flow rate (mL/d) and V the culture volume (mL). Under a flow condition utilized in the present study, $Q = 60$ mL/h and $V = 5500$ mL so that $k' = k\ (1 - Q/(Q + V)) = 0.7925\,k$. This loss of sensitivity associated with the continuous flow design can be compensated for by increasing the cell count correspondingly and thus the detection limit, but it must still be considered as a cost associated with the greater reality of continuous flow versus batch cultures.

The rate of change of the cell concentration of a species in continuous culture is described by the differential equation (equation 3) (Rich 1973):

$$(3)\quad \frac{dC_1}{dt} = \frac{Q}{V} C_0 - \frac{Q}{V} C_1 + k\, C_1$$

this relates the rate of change of the culture concentration (dC_1) to the flow (Q/V), inflow concentration (C_o), culture concentration (C_1), and growth rate (k). The state of a culture after a given time interval can be computed through integration. Rich (1973) provides a general solution as well as specific solutions for conditions of constant inflow concentration as well as linearly or exponentially increasing inflow concentration. This complexity is entirely avoided by comparison of experimental cultures receiving perturbation with nonmanipulated control cultures which integrate inflow effects. The only effect of flow on such comparisons is to reduce the sensitivity, as noted above.

Results

Enclosure Effects

Variable flow rates, particularly for cultures of the same experiment, can be one source of enclosure effect as indicated by the response detection calculations presented above. In operation both laboratory and in situ cultures remained within $2\ mL \cdot h^{-1}$ of each other and within $\pm 2\ mL \cdot h^{-1}$ of the set rates of 50 or $60\ mL \cdot h^{-1}$.

Instead of attempting to isolate and evaluate all possible conditions leading to enclosure effects, the similarity of control culture compared to lake communities was taken as a measure of the composite of all enclosure effects. The four similarity indices each lead to the same conclusions for both the epilimnion and hypolimnion communities used in experiments 1 and 2 (Tables 1, 2). In situ cultures which all received unfiltered lake water maintained communities most like those in the lake, followed by lab cultures which received unfiltered lake water. The lab cultures which received filtered lake water were least like the lake. Many species declined in the latter treatment, indicating that filtered inflow not balanced by filtered outflow was depleting these species in the enclosed community. The similarity between replicate lake samples (Tables 1 and 2, "Lake") was compared to the similarity between lake and culture samples Tables 1 and 2, "Lake vs. in situ" or "lake vs. lab") using T tests. At the end of experiment 1 the similarity between replicate lake samples differed from the similarity between those lake samples and the lab culture (filtered or unfiltered

TABLE 1. Similarity of communities for experiment 1 from the epilimnion of Lake 239 using two in situ and four lab (two receiving raw water-R, two receiving filtered water-F) continuous cultures. Similarity between culture and lake communities for 25 species using four indices of similarity (see text) with each value the mean of all possible sample pair comparisons (#). Standard error of the mean is given after each mean similarity. Total biomass for the 25 species as $mg \cdot L^{-1}$. Comparisons at the beginning and after 144 h. (*Beginning samples clustered from 144 h.)

	SIMI	%S	Cb*	Cb%*
Initial				
Lake vs. in situ (1)	99.56	92.90	5.62	5.91
Lake vs. lab R (2)	99.30(0.19)	90.48(1.40)	6.23(0.95)	6.51(0.92)
Lake vs. lab F (2)	98.45(6.14)	88.20(2.79)	7.80(0.09)	7.74(0.02)
Biomass	lake — 0.395	in situ — 0.412	lab R — 0.426	lab F — 0.386
144 h				
Lake (3)	99.46(0.07)	91.35(0.41)	3.99(0.26)	5.18(0.04)
Lake vs. in situ (3)	97.67(0.46)	83.36(1.53)	5.14(0.16)	6.36(0.35)
Lake vs. lab R (6)	96.77(0.30)	80.08(0.71)	7.82(0.18)	7.35(0.19)
Lake vs. lab F (6)	92.97(0.29)	76.60(0.67)	7.54(0.38)	8.02(0.21)
Biomass	lake — 0.403	in situ — 0.324	lab R — 0.563	lab F — 0.187

TABLE 2. Similarity of communities for experiment 2 from the hypolimnion of L226 SW using two in situ and four lab (two controls, one increased P, one increased light) continuous cultures. Similarity between culture and lake communities for 21 species using four indices of similarity (see text) with each value the mean of ail possible sample pair comparisons (#). Standard error of the mean is given after each mean similarity. Total biomass for the 21 species as $mg \cdot L^{-1}$. Comparisons at the beginning and after 267 h. (*Eight beginning samples clustered separately from eight 267 h samples.)

	SIMI	%S	Cb*	Cb%*
Beginning				
Lake (1)	99.41	94.69	4.70	3.27
In situ (1)	99.99	98.66	5.48	4.69
Lab C (1)	99.96	97.96	3.35	4.33
Lake vs. in situ (4)	99.72(0.14)	96.10(0.92)	5.81(0.29)	4.85(0.29)
Lake vs. lab C (4)	96.50(0.81)	85.04(1.38)	6.76(0.23)	7.95(0.66)
Lake vs. lab P (2)	96.31(1.42)	85.15(2.28)	7.55(0.05)	7.73(0.31)
Lake vs. lab L (2)	96.67(1.33)	85.25(2.40)	8.04(0.84)	7.33(0.41)
Biomass lake — 2.998	in situ — 2.784	lab C — 2.531	lab P — 2.439	lab L — 2.711
267 h				
Lake (1)	99.92	96.65	4.18	4.51
In situ (1)	99.94	97.14	2.37	3.03
Lab C (1)	99.28	92.58	4.87	5.52
Lake vs. in situ (4)	99.02(0.16)	91.49(0.70)	5.10(0.24)	5.20(0.19)
Lake vs. lab C (4)	96.45(0.86)	82.26(2.09)	7.40(0.40)	7.76(0.51)
Lake vs. lab P (2)	96.11(0.36)	80.85(1.47)	7.93(0.66)	8.04(1.12)
Lake vs. lab L (2)	94.83(0.41)	76.90(2.04)	8.77(0.67)	8.60(1.29)
Biomass lake — 3.949	in situ — 3.584	lab C — 4.635	lab P — 4.507	lab L — 5.383

inflow) at the 0.001 level. The levels of significant difference for the four indices were averaged to obtain this value. The same comparison with the in situ cultures showed the similarities to be less significantly different, being at the 0.05 level. At the end of experiment 2 the similarity between replicate lake samples differed from the similarity between those lake samples and the lab control cultures at the 0.1 level, and from the in situ cultures at the 0.2 level.

At the end of both experiments the total algal biomass of the lab cultures diverged more from the lake than the in situ cultures did (Tables 1 and 2). In

TABLE 3. Comparison of species biomass for experiment 2 from the hypolimnion of L226 SW. Biomass of each species expressed as $10^3 \mu m^3 \cdot mL^{-1}$ ($\times 10^{-3}$ for $mg \cdot L^{-1}$) as means using two lake samples and single samples from two in situ and four lab (two controls, one increased P, one increased light) continuous cultures. Comparisons after 267 h.

	Lake	In situ controls	Lab controls	PO_4-P	Light
Anabaena sp.	23.4	26.8	16.5	14.3	19.2
Ankistrodesmus falcatus	15.7	14.3	16.5	16.8	16.2
Botryococcus braunii	39.0	41.6	34.5	38.2	39.0
Ceratium hirundinella	139.8	47.0	0	0	0
Chrysosphaerella longispina	802.8	546.4	430.2	372.6	307.7
Coelosphaerium sp.	2.5	2.9	3.8	3.7	4.0
Cosmarium sp. A	3.1	4.4	6.8	5.2	3.9
Cosmarium sp. B	22.9	23.6	32.0	28.6	32.3
Cryptomonas erosa	95.7	77.3	88.8	64.8	66.6
Cryptomonas obovata	34.9	21.3	27.0	29.9	28.5
Cryptomonas sp.	6.5	4.0	3.3	2.0	1.8
Cyclotella glomerata	53.1	48.9	59.8	55.9	71.2
Cyclotella sp.	40.0	42.0	44.1	44.1	60.5
Dinobryon bavaricum	4.5	3.7	4.5	5.4	5.0
Dinobryon sertularia	654.7	602.0	900.1	861.4	1097.2
Mallomonas caudata	21.8	14.9	18.3	11.2	9.8
Mallomonas pumilio	39.1	34.8	20.7	22.3	18.4
Scenedesmus bijuga	45.6	49.7	69.1	68.5	58.2
Synedra radians	44.5	43.6	71.6	120.2	60.5
Synura uvella	1809.3	1879.7	2759.9	2722.2	3463.3
Tabellaria flocculosa	50.1	54.6	27.5	20.1	19.4
Total	3949.0	3583.5	4635.0	4507.4	5382.7

experiment 2, comparing the biomass of each species after 380 h (Table 3), 16 of the 21 species had values in the in situ cultures most like those in the lake.

Enclosure effects from experiment 3, where only in situ cultures were used, were evaluated by plotting the distribution of four species in two control cultures and two lake samples through time. For *Dinobryon sertularia* and *Elakatothrix* sp., no culture values were significantly different ($P < 0.05$) from the lake (Fig. 3, 5). For *Rhabdoderma gorskii* and *Dinobryon bavaricum*, on two of the four sampling dates differences were significant at $0.025 < P < 0.05$.

RESPONSES TO PERTURBATIONS

In experiment 2, the biomass of *Synedra radians* increased (significant at $P < 0.05$) in the culture with elevated P. *Synura uvella*, *Dinobryon sertularia*, *Cyclotella glomerata*, and *Cyclotella* sp. increased (significant at $P < 0.05$) in the culture with elevated light (Table 3). These responses, including C fixation rates for individual species, are described in greater detail in Knoechel and deNoyelles (1980). In spite of the significant responses for certain species, the community similarities showed only slight declines with perturbation (Table 2). At the end of the experiment the similarity between lake and laboratory control cultures differed from the similarity between lake and the P culture only at the 0.9 level, and with lake and the high light culture at the 0.5 level. Similarity values from treatment cultures compared to laboratory control cultures (not given in Table 2) led to the same conclusion. All laboratory cultures in experiment 2 showed an increase in total biomass compared to the in situ cultures or to the lake (Table 3). The greatest increase occurred with increased light. As it is impossible to exactly simulate in situ light in the laboratory, some increases in all the other laboratory cultures may have been due to higher light. In spite of this the distributional responses of the species cited above were significant ($P < 0.05$) as related to the perturbations of elevated P and elevated light, as were responses in terms of carbon fixation rates (Knoechel and deNoyelles 1980).

Responses to perturbation in experiment 3 are distinguishable for phytoplankton exposed to a Cd concentration between 2.9 and 4.2 $\mu g \cdot L^{-1}$. Only species also common in the tube experiment from L223 were considered, since we wished to compare continuous culture and tube responses. For the in situ continuous cultures, *Dinobryon bavaricum* (Fig. 2) declined to a third of its control level after only 70 h of Cd exposure. *Elakatothrix* sp. (Fig. 3) and *Rhabdoderma gorskii* (Fig. 4) during the same time showed little change over the controls but then increased by the end of the continuous flow period with Cd of 188 h. Another species of *Dinobryon* (Fig. 5), not common in the tubes, responded similarly to *D. bavaricum*. For both species of *Dinobryon* the declines were significant

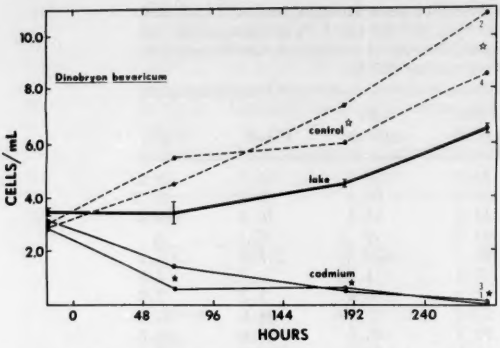

FIG. 2. Changes in abundance as cells · mL^{-1} for *Dinobryon bavaricum* from experiment 3 with 4 in situ continuous cultures operated in L239, 2 as controls (#2, #4) and 2 with Cd ranging in concentration between 2.9 and 4.2 μg · L^{-1} (#1, #3). Abundance in the lake is expressed as the mean of two samples with the range indicated. Cd was first added at 0 h, 20 h after incubation and flow was begun, and then once a day for the next 188 h, after which flow was terminated and incubation continued for another 94 h. Solid stars indicate treatment values that are significantly different from controls and open stars indicate control values that are significantly different from the lake ($P < 0.05$).

($P < 0.05$) at 70 h and beyond, and for *E.* sp. and *R. gorskii* the increases were significant ($P < 0.05$) at 188 h and beyond. Comparing the culture responses to those in the tubes, the same three species responded in a similar way over a wider range of Cd concentrations (Table 4). *Dinobryon bavaricum* declined below lake and control tube numbers when exposed to a concentration as low as 1 μg Cd · L^{-1}. *Elakatothrix* sp. and

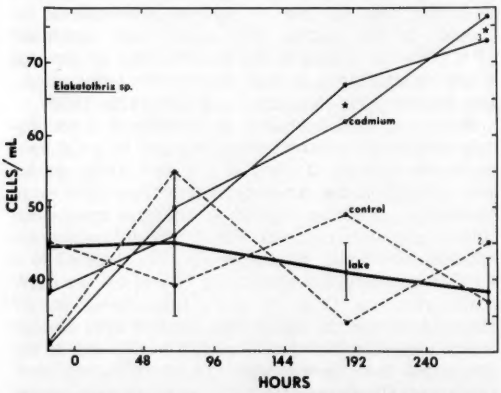

FIG. 3. Changes in abundance as cells · mL^{-1} for *Elakatothrix* sp. from experiment 3 in L239. See Fig. 2 legend for details.

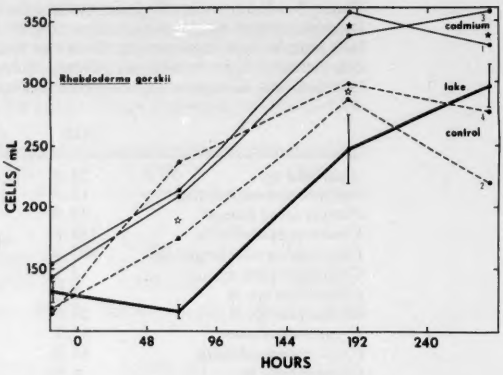

FIG. 4. Changes in abundance as cells · mL^{-1} for *Rhabdoderma gorskii* from experiment 3 in L239. See Fig. 2 legend for details.

R. gorskii remained the same or increased when exposed to all Cd concentrations, the highest being 30 μg Cd · L^{-1}.

Perturbations in the form of increased light, P, and Cd caused rapid and significant biomass or C uptake responses at the species or community level in the cultures compared to both the lake and control cultures. It remains to be considered how realistic these responses are, and therefore how accurately they can be extrapolated to whole lakes. Here too the influence of the unnatural conditions which even the in situ continuous cultures impose upon the organisms must be considered.

Discussion

The conditions considered by investigators as causing enclosure effects for phytoplankton have been reviewed by Venrick et al. (1977). Of particular importance are unnatural physical conditions involving

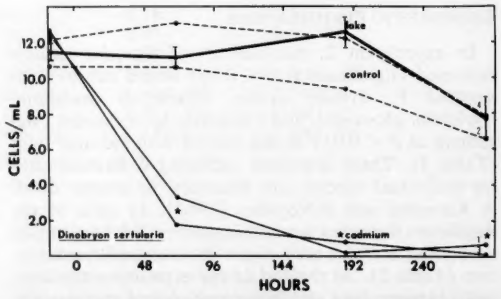

FIG. 5. Changes in abundance as cells · mL^{-1} for *Dinobryon sertularia* from experiment 3 in L239. See Fig. 2 legend for details.

TABLE 4. Comparison of the abundance of three species for a tube experiment from Lake 223. Cadmium was added once to each tube at the beginning of the experiment at concentrations of 1, 3, 10, and 30 μg Cd·L^{-1}. Species abundance expressed as cells·mL^{-1} using one sample from each tube and the lake taken at a depth of 1 m. Comparisons after 14 d.

	Dinobryon bavaricum		*Rhabdoderma gorskii*		*Elakatothrix* sp.	
	Beginning	14 d	Beginning	14 d	Beginning	14 d
Lake	23.0	11.3	150	196	100	116
Control	7.6	84.0	73	175	65	74
1	4.5	1.5	56	262	35	124
3	15.6	0.2	174	208	76	92
10	17.8	0.9	106	190	33	84
30	15.9	1.9	129	318	86	67

light, temperature, turbulence and handling of the organisms, and unnatural chemical conditions involving nutrient depletion and induced homogeneity. Responses to enclosure can begin with physiological ones and then develop into altered species distribution.

Attempts to minimize enclosure effects are numerous. In situ incubation for all types of enclosures produces better replication between control cultures and the lake community. This was demonstrated for continuous culturing in the present study. With another type of enclosure, batch cultures of a few 100 mL in size, further improvement is attempted by using a short incubation period of a few hours (Soeder and Talling 1969; Strickland and Parsons 1968; deNoyelles and O'Brien 1974). The intent is to allow insufficient time for nutrients to become depleted (Eppley 1968) or for physical conditions to have an effect. Such enclosures are known to produce physiological responses to enclosure during the first 24 h of incubation (Pratt and Berkson 1959; Verduin 1960; Venrick et al. 1977). Such responses, often involving rates of photosynthesis, can become even more pronounced over several days of incubation (Menzel et al. 1963). Changes in species distribution as caused by this containment have also been reported (Pratt and Berkson 1959; Sheldon et al. 1973). Venrick et al. (1977), working with phytoplankton from oligotrophic ocean waters, recorded both physiological and distributional changes within 24 h. Though short incubation may solve some problems, it does not allow time for initial growth responses such as those measured by C uptake to develop into measurable changes in species distribution which can provide additional information about the response to perturbation. Furthermore, some initial growth responses may not accurately represent those to follow (Knoechel and deNoyelles 1980). This can be due to a lag period preceding an organism's change in C uptake following the first exposure to perturbation (Gerhart and Likens 1975; O'Brien and deNoyelles 1976; deNoyelles and O'Brien 1978; Knoechel and deNoyelles 1980).

Increasing the size of the enclosure provides other improvements, particularly for tubes (Goldman 1962; Lund 1972; Schindler et al. 1971, 1974; Takahashi et al. 1975), which can also be left open to natural exchanges with the atmosphere and sediments, particularly of nutrients. Conditions such as temperature and light which change with depth can also be incorporated into the large enclosure. This is not possible with any of the smaller enclosures confined to one depth. Increased size reduces the surface to volume ratio, which should also reduce suggested effects such as stimulation of bacterial growth (Pratt and Berkson 1959), which could alter nutrient recycling. Providing natural turbulence remains a problem even in the largest enclosures (Lund 1972) since they are still isolated from most of the mixing being generated in the lake.

The continuous culture using our method of laboratory or in situ operation is intended to provide a compromise between the large, open, more encompassing conditions of tubes and the small, isolated though more convenient batch bottle cultures. Nutrient renewal in continuous culture helps to extend the incubation time so that perturbation responses such as changes in community structure can be sought. Though it receives inflow from one depth, the enclosed community is continually renewed with organisms which have been circulating naturally in the lake. This same renewal displaces organisms which may be developing enclosure responses and provides new organisms to be exposed to perturbation; for in situ incubation this occurs immediately upon their leaving the natural environment. For the in situ continuous cultures relocation of organisms to the enclosure occurs rapidly and so causes less of an initial disturbance to them compared to filling batch cultures (Venrick et al. 1977) or laboratory continuous cultures. Continual inputs from the surroundings in both types of continuous cultures may also compensate for a possible consequence of the hypothesis of contemporaneous disequilibrium (Hutchinson 1961; Richerson et al. 1970). This hypothesis attempts to explain the high natural species diversity in phytoplankton communities as a consequence of a heterogeneous environment even in a well mixed epilimnion. If this is true, the more homogeneous conditions of a batch culture should favor the competitive exclusion of some species. Though large tubes contain some of the natural heterogeneity of physical, chemical,

and biological conditions, the continuous culture continually draws from it. Finally, even with renewal of organisms there is still time during their residence for responses to perturbation to develop which can be detected above the accompanying dilution, as demonstrated here with light, P, and cadmium.

In the present study the species distribution of the phytoplankton in control cultures incubated in the laboratory or in situ remained similar to that of the lake community (Tables 1–3). This indicates a reduction of enclosure effects. The highest degree of similarity was maintained by in situ incubation, thus the greatest potential for extrapolation would likely be found here. However, as in the case of experiment 2 with light, it is not always possible to apply the perturbation in situ. Still the lab cultures were able to provide responses of the organisms consistent with the finding of other investigators. Fee (1976) demonstrated the common occurrence of a phytoplankton biomass peak (Fee et al. 1978) in the upper hypolimnion of several oligotrophic lakes at the ELA similar to the one we studied in SW L226. He considered that the algae may be making a compromise between adequate nutrients and light, thus being at or close to a limiting condition for both. Therefore, some species should be responsive to nutrient or light increases, as was observed during experiment 2 (Table 3) and is reported on in greater detail by Knoechel and deNoyelles (1980).

Tube experiments, which are less likely to have some container effects, from L223 provided an evaluation of the in situ culture responses to Cd for the phytoplankton community of L239. Though only three species were abundant and recorded in both experiments they showed similar trends of biomass increases or decreases during the second week of exposure (Fig. 2–4, Table 4). Increased biomass of *Elakatothrix* sp. and *Rhabdoderma gorskii* in the presence of Cd probably resulted from some form of competitive release rather than a direct stimulus. Particularly in these oligotrophic waters where nutrients are often limiting, exposure of species resistant to Cd may experience less competition for nutrients.

Although tubes had been set in place just a few days, numbers of the three species differed from each other and the lake at the beginning of the experiment, indicating some early enclosure effects. Differences between the lake and the control tube continued through day 14 (Table 4). However, two types of enclosures operated in different lakes, both produced similar responses from the same species. They also demonstrated how different the responses to Cd can be for different species within the same community.

Considering the various types of enclosures available for work with phytoplankton, the nature of the questions being asked must figure into the choice of method. Where the responses sought must develop over at least a few days, such as species succession, then minimizing container effects becomes of utmost importance. Only large enclosures such as bags and tubes or our continuous cultures have proven adequate for such purposes. More immediate responses such as the first few hours of C uptake for a community or species may be sought in the small batch containers. However, the immediate responses of the phytoplankton may not be the same as or predictive of their subsequent longer-term responses. Continuous cultures can produce these longer-term responses and, without perturbation, can maintain the natural community structure for the necessary comparisons. Measurement of short-term carbon uptake responses for individual species can be added to the operation of continuous cultures (Knoechel and deNoyelles 1980). This increases the sensitivity for detecting a species response in the culture while allowing carbon uptake to be followed as a long-term response.

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Analysis of the Response of Hypolimnetic Phytoplankton in Continuous Culture to Increased Light or Phosphorus Using Track Autoradiography

R. KNOECHEL[1]

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

AND F. DENOYELLES JR.

Department of Systematics and Ecology, University of Kansas, Lawrence, KS 66045, USA

KNOECHEL, R., AND F. DENOYELLES JR. 1980. Analysis of the response of hypolimnetic phytoplankton in continuous culture to increased light or phosphorus using track autoradiography. Can. J. Fish. Aquat. Sci. 37: 434–441.

Phytoplankton from the hypolimnetic biomass peak in Lake 266SW (Experimental Lakes Area, northwestern Ontario) was subjected to conditions of increased light or phosphorus in continuous culture. Phosphorus enrichment increased total carbon fixation 16% relative to the control; however, the particulate fraction was initially depressed and the phytoplankton biomass was less than 2% greater after 10 d. The high light culture displayed 21% higher final biomass despite a 13% decline in total carbon fixation which was primarily due to reduced extracellular release. The 21 most common species demonstrated a wide range of positive and negative biomass responses to the experimental perturbations; however, the three dominant chrysophyte species all responded positively to higher light. Detection of population biomass changes provided a direct and accurate means of quantifying perturbation effects provided the species response was stable over several days. Measurement of carbon uptake rates of species through track autoradiography provided the additional sensitivity and temporal resolution needed to monitor low level or variable responses. *Synedra radians* exhibited a lengthy lag period before demonstrating a strong positive response to phosphorus enrichment, while *Dinobryon sertularia* shifted from neutral to strongly negative and then strongly positive within a 3-d period. These results illustrate the inadequacy of short-term bioassays for the prediction of long-term perturbation effects.

Key words: growth rate, primary production, *Ankistrodesmus falcatus*, *Dinobryon sertularia*, *Synedra radians*

KNOECHEL, R., AND F. DENOYELLES JR. 1980. Analysis of the response of hypolimnetic phytoplankton in continuous culture to increased light or phosphorus using track autoradiography. Can. J. Fish. Aquat. Sci. 37: 434–441.

Les auteurs ont soumis du phytoplankton prélevé au moment du maximum de biomasse hypolimnétique dans le lac 266SW (région des lacs expérimentaux, nord-ouest de l'Ontario) à des conditions croissantes d'éclairage ou de phosphore en culture continue. L'enrichissement au phosphore augmente la fixation du carbone de 16% par rapport au témoin; cependant, la fraction particulaire décroît initialement et la biomasse phytoplanctonique, après 10 jours, n'augmente que de 2%. La culture soumise au fort éclairage produit une biomasse finale de 21% plus grande, en dépit d'un déclin de 13% de fixation de carbone total, déclin dû surtout à une mise en liberté extracellulaire moindre. Les 21 espèces les plus communes ont une gamme étendue de réponses, biomasses positive et négative, aux perturbations expérimentales; cependant, les trois espèces dominantes de chrysophytes réagissent toutes positivement à un fort éclairage. La détection des changements de biomasse dans la population constitue un moyen direct et précis de quantification des effets de ces perturbations, pourvu que la réponse des espèces ait été stable depuis plusieurs jours. La mesure des taux d'absorption du carbone par les espèces au moyen de l'autoradiographie de trace fournit la sensibilité supplémentaire et la résolution temporelle nécessaires à la surveillance de réponses variables ou de faible intensité. Ce n'est qu'après un long décalage que *Synedra radians* accuse une forte réponse positive à l'enrichissement au phosphore, alors que *Dinobryon sertularia*, sur une période de 3 jours, passe de la condition neutre à fortement négative et ensuite à fortement positive. Ces résultats démontrent l'insuffisance d'essais biologiques à court terme dans la prédiction des effets à long terme des perturbations.

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[1]Present address: Department of Zoology, Erindale College, University of Toronto, Mississauga, Ont. L5L 1C6.

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THE clear Precambrian Shield waters of the Experimental Lakes Area (ELA) typically support summer hypolimnetic phytoplankton biomasses exceeding those of the epilimnia (Fee 1976). The hypolimnetic communities are characterized by the strong dominance of a few chrysophyte flagellate species and by the steady accumulation of biomass throughout the summer (Fee et al. 1977; Fee 1978). Short-term incubator experiments have indicated that hypolimnetic photosynthesis is light limited (Fee 1976) whereas long-term hypolimnetic phosphorus addition has produced higher biomass, suggesting phosphorus limitation (Fee 1976; Schindler et al. 1980). In the present study we investigate the response of individual hypolimnetic bloom species and of the entire community to increased levels of either phosphorus or light under controlled laboratory conditions using continuous cultures with periodic monitoring of population size and carbon uptake rates of species. The results permit a comparative evaluation of short-term versus long-term bioassay approaches.

Materials and Methods

Continuous cultures were established in the laboratory with raw lake water collected from the hypolimnetic biomass peak community at 6.5 m in Lake 226SW (see deNoyelles et al. 1980) on August 7, 1978, and were maintained at displacement rate of 26%/d (60 mL·h^{-1} into a 5.5-L culture) with unfiltered water collected at 2-d intervals from the same depth. The cultures were maintained at 10°C illuminated by cool white fluorescent lamps on a 13 h:11 h light–dark cycle with 15 μEi·m^{-2}·s^{-1} photosynthetically available radiation at the culture vessel surface; conditions were chosen to approximate those of the natural community. Two cultures were maintained as controls (only one of which is reported herein); one was exposed to twice the control incident radiation and another was enriched with phosphorus (as phosphoric acid) initially added as a spike to raise the natural concentration 10.0 μg·L^{-1} and then pumped from a separate reservoir to increase influent waters by 20 μg·L^{-1}. The continuous addition of raw lakewater effectively replicates each culture continuously because new organisms are constantly being introduced to the experimental conditions (cf. deNoyelles et al. 1980). The experimental run was terminated at 10 d, all times being referenced to the initiation of P enrichment.

Numerical responses of species were monitored through enumeration of acid Lugol's preserved samples (Vollenweider 1974) on an inverted microscope using the Utermöhl (1958) technique. Significant differences between treatments and control on a given day were detected by subjecting paired 100-field counts from each culture to a model I analysis of variance (ANOVA, Sokal and Rohlf 1969) with a priori planned comparisons between each treatment and the control.

Photosynthetic rates were determined by incubating subsamples withdrawn from each chemostat for 2 h in 125-mL pyrex BOD bottles with from 1.5 to 3.7·10^5 Bq (1 Ci = 37 GBq) ^{14}C–$NaHCO_3$. Total dissolved inorganic carbon was measured in each sample by means of a conductimetric technique (Stainton et al. 1977). Available ^{14}C was determined separately for each sample at the end of incubation by counting a 3-mL aliquot in 10 mL of scintillation fluid (PCS, Amersham) to which 1 mL of pH 10 buffer had been added to avoid loss of carbon dioxide, following which the remainder of each sample was preserved with acid Lugols solution. Total community ^{14}C uptake was estimated from scintillation counts of triplicate acidified and bubbled sample aliquots (Schindler et al. 1972) and was further subdivided into particulate and dissolved fractions through a passive settling technique (R. Knoechel and J. Kalff unpublished tech.). Statistically significant differences were detected by a one way ANOVA with planned comparisons between treatments and control.

The ^{14}C uptake rates of individual species were determined through track autoradiography, a technique whereby a thick layer of photographic emulsion visibly records the path of beta particles leaving individual cells, allowing disintegration rates to be determined at the species level (Knoechel and Kalff 1976a). Tracks were counted over a minimum of 100 cells of each species of interest from each sample. In the case of the colonial flagellate, *Dinobryon sertularia*, 20 colonies comprising a total of over 100 cells were analyzed. Track counts for a species were converted into cell carbon fixation rates by correcting for the π geometry of the emulsion detector and for self-absorption (Knoechel and Kalff 1976a, b) as well as for the number of cells counted, the length of slide exposure, and the ^{12}C:^{14}C ratio. The latter three factors were used to calculate the expected distribution of tracks between slides from treatment and control chemostats for each species, assuming no difference in actual cellular carbon fixation rate. This null hypothesis was then tested by comparing the absolute value of the difference between expected and observed track counts on one slide to the standard deviation for the expected binomial distribution (Wetherill 1967) thus:

$$z = \frac{|pk - x| - 0.5}{(kpq)^{1/2}}$$

where: p = expected proportion of tracks on slide #1
q = expected proportion of tracks on slide #2 = $1 - p$
k = total tracks slides 1 plus 2
x = tracks observed on slide #1
0.5 = correction factor

The probability of obtaining an equal or greater value for the statistic z was determined by consulting a table of areas under the normal curve (two-tailed test, Rohlf and Sokal 1969).

Species cell volumes, determined from dimensions obtained with a calibrated ocular micrometer using equations for simple geometric solids, were used to calculate carbon fixation rates per unit of biomass thereby facilitating comparisons between species ranging widely in size.

A Relative Activity Index (RAI) was devised from species biomass and carbon uptake data expressed as percentages of total community biomass and community particulate carbon fixation:

$$\text{RAI} = \%\ \text{community production}/\%\ \text{community biomass}$$

The community as a whole must by definition have an RAI equal to 1.00. Species populations with RAI values greater than unity are more productive than average on a per unit volume basis. Such species should either be expanding relative to the rest of the community or else should have higher than average carbon content per unit volume and/or higher than average losses such as death and respiration to offset the apparent growth advantage. Simi-

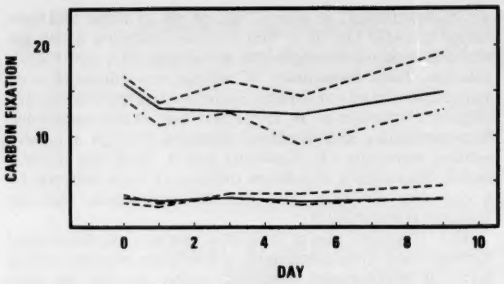

FIG. 1. Phytoplankton community carbon fixation ($mg \cdot m^{-3} \cdot h^{-1}$) in high light (·····), control (———) and phosphorus enriched (- - - - -) continuous cultures. The upper set of lines represents total carbon fixation while the lower set is the particulate fraction, the difference being the extracellular release fractions.

larly, populations with RAI values less than unity should either be declining in relative abundance or else be subject to lower than average losses and/or have lower than average carbon contents.

Results

COMMUNITY

Experimental manipulation of light and phosphorus produced changes in community biomass levels which were considerably at odds with the observed changes in community carbon fixation. Phosphorus enrichment produced a significant increase (ANOVA, $P < 0.05$) in total carbon fixation relative to the control at all times; however, particulate carbon fixation was significantly lower (ANOVA, $P < 0.05$) on day 0 and day 1 following which it was significantly higher ($P < 0.05$) throughout the remainder of the experiment (Fig. 1). Despite this, there was less than a 2% difference in total phytoplankton biomass noted on day 10 (Table 1). In contrast, the high light culture demonstrated at 21% higher biomass than the control culture at that time despite particulate carbon fixation rates which never significantly exceeded those of the control (Fig. 1). Total carbon fixation was significantly lower (ANOVA, $P < 0.05$) than the control on all dates, due largely to a reduced level of extracellular release. Particulate fixation was significantly lower ($P < 0.05$) only on day 1 and day 5.

Particulate carbon fixation averaged 24.2, 21.8, and 20.7% of total carbon fixation in the high light, control, and phosphorus enriched cultures, respectively, (unweighted arithmetic average) consistent with high levels of extracellular release expected for communities growing at extremely low light levels (see Nalewajko and Schindler 1976).

SPECIES

Initial and final biomass estimates for the 21 most common species permitted an analysis of the effect of treatment on final yield (Table 1). This in turn was the basis for selecting three species representative of different response types which were examined in greater detail through monitoring of population size and carbon uptake rate throughout the experimental run. Species selected were: *Synedra radians*, a diatom which displayed the greatest positive biomass response to phosphorus addition; *Dinobryon sertularia*, one of the dominant chrysophyte flagellates which showed a positive biomass response to light (*Synura* was not chosen because of difficulty in distinguishing it from *Chrysosphaerella* on the autoradiography slides) and *Ankistrodesmus falcatus*, a nonmotile green alga whose biomass level did not respond to either treatment and thus provided an additional test for agreement between the two techniques.

FINAL BIOMASS

Phosphorus enrichment and increased light each produced increased biomass of some species and decreased biomass of others. To detect treatment effects, all biomass changes must be referenced to the control culture to distinguish the effect of adding raw lakewater. Increased light produced 5 positive and 4 negative biomass responses while phosphorus enrichment produced 2 positive and 3 negative responses (Table 1, ANOVA, $P < 0.10$).

Effects of treatment were not uniform among species of the same taxonomic division; however, increased light did produce a positive biomass response in each of the three dominant species: *Chrysosphaerella multispina*, *Dinobryon sertularia*, and *Synura sphagnicola*. These chrysophyte species are typical dominants of hypolimnetic blooms at ELA (Fee et al. 1977; Fee 1978) and elsewhere in North America (deNoyelles and Knoechel personal observations). It should be noted that all species responding to phosphorus also responded to light, although the effects were sometimes in the opposite direction (Table 1).

RESPONSE OF *Synedra radians*

Phosphorus enrichment produced strongly positive carbon uptake and numerical responses in this species while increased light produced generally negative effects.

There was a lag in response to phosphorus enrichment with the first significant difference in carbon uptake being noted on day 3 when the rate was 49% higher than that in the control culture (Fig. 2, $P < 0.001$). The differential increased to 63 and 172% of the control on days 5 and 9, respectively ($P < .001$). The numerical response mirrored that of carbon uptake but with a greater lag period. Cell counts 12, 29, and 72% higher than the control were noted on days 4, 6, and 10, respectively (Fig. 2: $P < 0.05$, $P < 0.01$, $P < 0.001$). The area under the carbon fixation versus time curve (Fig. 2), a time integrated measure of pro-

TABLE 1. Initial and final population biomass of 21 most common species in high light, control, and phosphorus enriched cultures. A "+" or "−" response indicates that the final biomass in the treatment culture is significantly higher or lower than the control ($P < 0.05$, ANOVA paired 100-field cell counts).

Species	Volume (μm^{-3})	Initial biomass ($mg \cdot m^{-3}$)			Final biomass ($mg \cdot m^{-3}$)			Response	
		+Light	Control	+P	+Light	Control	+P	+Light	+P
Anabaena sp. (mm)	12 560	11.3	11.9	15.1	19.2	14.2	14.3		
Ankistrodesmus falcatus	32	12.0	15.3	13.0	16.2	16.6	16.8		
Botryococcus Braunii (col.)	4 187	27.0	34.5	39.0	39.0	34.5	38.2		
Ceratium hirundinella	80 000	22.0	16.0	8.0	0.0	0.0	0.0		
Chrysosphaerella multispina (col.)[a]	20 933	510.8	414.5	420.8	307.7	265.8	372.6	(+)[b]	+
Coelosphaerium sp.	628	3.1	4.0	4.0	4.0	3.2	3.7	+	(−)
Cosmarium sp.	1 808	5.2	5.8	4.2	3.9	5.8	5.2		
Cosmarium sp.	251	20.1	23.3	21.5	32.3	37.3	28.6	−	−
Cryptomonas erosa	804	70.6	63.0	54.3	66.6	81.2	64.8		
Cryptomonas obovata	7 948	14.2	11.4	12.8	28.5	22.7	29.9		
Cryptomonas sp.	196	11.1	9.7	8.4	1.8	3.3	2.0	−	(−)
Cyclotella glomerata	1 413	30.8	31.8	25.7	71.2	49.2	55.9	+	
Cyclotella sp.	25	29.8	29.4	27.9	60.5	58.6	44.1		
Dinobryon bavaricum	126	4.7	3.2	3.0	5.0	5.1	5.4		
Dinobryon sertularia	236	219.5	207.7	222.1	1097.2	858.6	861.4	+	
Mallomonas caudata	1 963	6.7	6.7	8.8	9.8	13.0	11.2		
Mallomonas pumilio	904	33.4	32.2	36.8	18.4	20.3	22.3		
Scenedesmus bijuga	42	67.3	68.8	69.7	58.2	68.4	68.5	−	
Synedra radians	360	28.0	26.9	28.2	60.5	70.2	120.2	−	+
Synura sphagnicola[c]	523	1550.7	1363.5	1386.0	3463.3	2808.5	2722.2	+	
Tabellaria flocullosa	1 408	31.5	32.2	31.0	19.4	26.9	20.1		
Community total		2709.8	2411.8	2440.3	5382.7	4463.4	4507.4		

[a]E.m. scale analysis: previously identified as *Chrysospharella longispina* using light microscope.
[b]Parentheses indicate response significant at $0.05 < P < 0.10$ level.
[c]E.m. scale analysis: previously identified as *Synura uvella* using light microscope.

duction, was 58% higher in the phosphorus enriched culture than in the control, comparing favourably with the final cell count which was 72% higher.

The high light *Synedra* population fixed carbon at a lower rate than the control on 4 of 5 occasions (Fig. 2) although the differences were only significant on days 1 and 3 ($P < 0.05$). There were no significant differences in cell number until the final count on day 10 which was 14% lower in the high light chemostat ($P < 0.05$), in general agreement with the time-integrated carbon fixation rate which was 19% lower.

The relative activity index (RAI) values for the *Synedra* populations indicate that they were considerably less productive than the community average. The high light and control culture populations maintained their relative abundance at slightly more than 1% of the community biomass despite RAI values averaging only 0.30 (Table 2) as compared to a community average of 1.00 (see methods). The phosphorus enriched culture had a RAI of 0.47 at the end of the experiment. Assuming similar loss rates and cell carbon contents in all three cultures, this higher value points toward further biomass expansion until the RAI is reduced to the control culture level.

The carbon fixation rates displayed by these hypolimnetic *Synedra* populations are similar to those noted for an epilimnetic population in Lac Hertel (Knoechel and Kalff 1978) and yield doubling time estimates of from 3.4 to 7.3 d. The average hourly carbon uptake rate in the phosphorus enriched culture from day 3 through 9 inclusive yields a daily production estimate of 7.0×10^{-5} g C·μm^{-3} biomass·d^{-1} when extrapolated to the entire light cycle. This is higher than the mean epilimnetic rate of 5.2×10^{-15} g C·μm^{-3} biomass·d^{-1} reported for the same species in Lac Hertel (Knoechel and Kalff 1978). In contrast, the average uptake rate in the control chemostat over the entire period was 3.9×10^{-15} g C·μm^{-3} biomass·d^{-1} while that in the high light culture was only 3.1×10^{-15} g C·μm^{-3} biomass·d^{-1}. These uptake rates are equivalent to the daily carbon renewal rates of 23% in the P enriched culture, 13% in the control, and 10% in the high light culture, assuming a cell carbon content equal to that estimated for the Lac Hertel population (31 ×

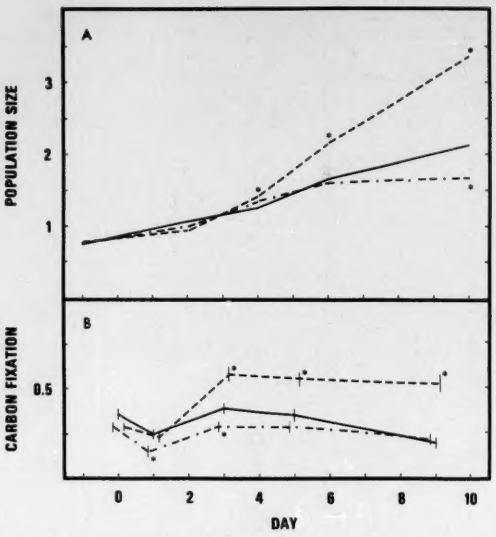

FIG. 2. Population size (A; cells·mL^{-1}) and carbon fixation rate (B; g C·10^{-15}·μm^{-3}·h^{-1}) for *Synedra radians* in high light (----), control (———), and phosphorus enriched (- - - -) continuous cultures. Vertical lines indicate standard error of carbon fixation rate and the treatment values are displaced to either side of the control to improve readability. Asterisks indicate treatment values that are significantly different from the control ($P < 0.05$).

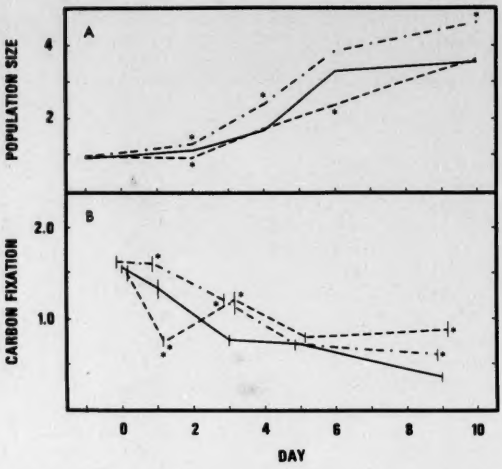

FIG. 3. Population size (A; cells·mL^{-1}) and carbon fixation rate (B; g C·10^{-15}·μm^{-3}·h^{-1}) for *Dinobryon sertularia* in high light (----), control (———), and phosphorus enriched) (- - - -) continuous cultures. Vertical lines indicate standard error of carbon fixation rate and treatment values are displaced to either side of the control to improve readability. Asterisks indicate treatment values that are significantly different from the control ($P < 0.05$).

10^{-15} g C·μm^{-3} biomass, Knoechel and Kalff 1978). These rates are equivalent to doubling times of 3.4, 5.8, and 7.3 d based on exponential growth (e.g. ln 2.0/ln 1.226 = 3.4).

RESPONSE OF *Dinobryon sertularia*

Increased light elicited a generally positive response from this community dominant while phosphorus enrichment produced variable effects on carbon fixation ranging from strong inhibition to strong enhancement.

Carbon fixation was initially unaffected by high light on day 0 following which significantly higher rates were noted on days 1, 3, and 9 (Fig. 3: $P < 0.05$, $P < .001$, $P < .05$) but not on day 5. This was reflected by significantly higher cell counts on days 2, 4, and 10 ($P < .025$, $P < .025$, $P < .005$) but not on day 6, demonstrating a short delay between changes in carbon fixation and cell number.

Phosphorus enrichment also had no effect on day 0 but then strongly inhibited carbon fixation on day 1 ($P < .001$) followed by significant stimulation on days 3 and 9 ($P < .001$). The early inhibition was reflected as a significantly lower cell count on day 2. This deficit was erased by the higher carbon fixation rates which followed; however, these rates were insufficient to further increase the cell counts significantly above the control. On day 6, cell number was significantly lower than the control in the phosphorus enriched culture, but this seems due to an anomalously high control cell count rather than to depressed carbon fixation (Fig. 3).

The control *Dinobryon sertularia* culture progressed from a state of high relative production to a low one during the experimental run. *Dinobryon* percent community production remained fairly stable in the control culture with a mean of 10.7% over the course of the experiment while percent community biomass rose from 8.6 to 19.2% and as a result the RAI declined from 1.31 to 0.49 (Table 2). In contrast, the percent community production doubled in the high light culture during the course of the experiment and increased rapidly in the phosphorus enriched culture late in the run so that final RAI values were 1.00 and 0.81, respectively (Table 2). This indicates considerable population expansion potential in both cultures assuming they both have cell carbon contents and loss rates similar to those in the control culture.

Carbon fixation rates declined on a per unit volume basis during the experimental period (Fig. 3). Daily rates ranged from a three culture mean of 20.2 × 10^{-15} g C·μm^{-3} biomass·d^{-1} on day 0 to a low of 4.4 × 10^{-15} g C·μm^{-3} biomass·d^{-1} in the control culture on day 9. Carbon renewal rates and doubling times ranged from 12.1% (6.1 d) for the three culture mean on day 0 to values of 2.65% (26.5 d), 4.67% (15.2 d),

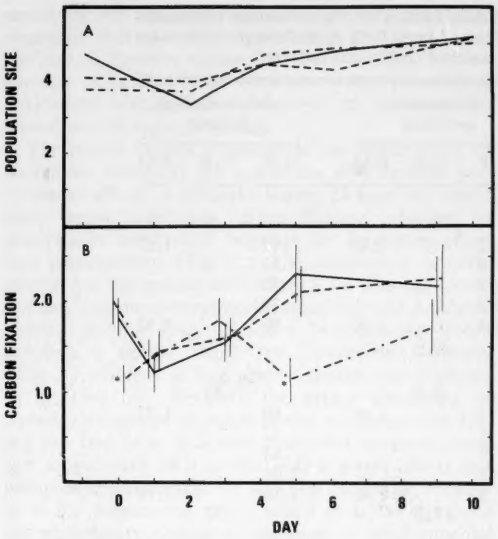

FIG. 4. Population size (A; cells·mL^{-1}) and carbon fixation rate (B; g C·10^{-15}·μm^{-3}·h^{-1}) for *Ankistrodesmus falcatus* in high light (·---·), control (———), and phosphorus enriched (----) continuous cultures. Vertical lines indicate standard error of carbon fixation rate and treatment values are displaced to either side of the control to improve readability. Asterisks indicate treatment values that are significantly different from the control ($P < 0.05$).

and 6.85% (10.5 d) for the control, high light and phosphorus enriched cultures respectively on day 9. These values are all calculated assuming a carbon content estimate of 167×10^{-15} g C·μm^{-3} biomass based on the regression equations of Strathmann (1967).

RESPONSE OF *Ankistrodesmus falcatus*

This small green alga showed little response to the experimental treatments. The only significant carbon uptake differences were inhibition in the phosphorus enriched culture on day 0 and in high light on day 5 (Fig. 4). Neither of these effects was large enough nor long-lasting enough to be reflected as a numerical change, there being no significant differences noted throughout the entire experiment (Fig. 4).

The *Ankistrodesmus* population declined in relative abundance in each of the cultures despite RAI values far above average. The RAI increased in the control culture from 1.27 at the beginning to 3.19 at the end with similar advances noted in both treatment cultures (Table 2). The large changes observed resulted more from major shifts in community parameters than from absolute changes in *Ankistrodesmus* production and biomass (Fig. 4).

Ankistrodesmus demonstrated a higher carbon fixation rate per unit biomass than either *Synedra* or *Dinobryon*. Carbon fixation averaged 23.3×10^{-15} g C·μm^{-3} biomass·d^{-1} in the control culture, equivalent to a carbon renewal rate of 10.7%·d^{-1} and a doubling time of 6.8 d assuming a carbon content of 218×10^{-15} g C·μm^{-3} biomass (Strathmann 1967). The maximum value, recorded on day 5, was 29.6×10^{-15} g C·μm^{-3} biomass·d^{-1} which is equivalent to a renewal rate of 13.6%·d^{-1} and a doubling time of 5.4 d.

Discussion

Although the effects of experimental manipulation on biomass varied widely at the species level the three dominant chrysophytes, which together comprised 84% of the total biomass, all displayed positive responses to light. The largest percentage biomass increase occurred in minor community components such as *Synedra radians* (Table 1) which doubled its biomass in the phosphorus enriched culture compared to the control but still contributed less than 3% of the community biomass. Dominant species such as *Synura sphagnicola*, *Dinobryon sertularia*, and *Chrysosphaerella multispina* were limited to more modest percentage gains (Table 1) even though they dominated the total community response due to their high abundance.

High light levels augmented biomass most effectively while production increased only with phosphorus enrichment. Total carbon fixation increased 16% in the phosphorus enriched culture relative to the control whereas in the high light culture it declined 13% (see Fig. 1). The inhibitory effect of high light was confined largely to the extracellular release fraction; however, particulate carbon fixation was only marginally lower (<2%) than in the control. Phytoplankton biomass increased only 1% in the phosphorus enriched culture as compared to 21% under high light, quite opposite to what one would predict based on community carbon fixation. Biomass failed to increase with phosphorus enrichment partly because of the initial biomass loss noted for *Dinobryon sertularia* (Fig. 3), one of the dominant species. Restitution of the loss by day 10 and the autoradiographic results both point towards an eventual biomass increase relative to the control (Fig. 3) in the phosphorus enriched culture.

The autoradiographic results reveal a redistribution of particulate carbon fixation at the species level. In the high light culture, the fixation rate of one of the dominant species, *Dinobryon sertularia*, increased relative to the control while that of a lesser component, *Synedra radians*, declined. This redistribution could produce increased community biomass in the absence of increased community production provided that the favoured species were subject to lower than average losses such as respiration, grazing and death as hypothesized by Fee (1976; cf. Kalff and Knoechel 1978). Alternatively, a similar result could be achieved by light affecting loss rates directly. From our results it is clear that high light significantly reduced one form of meas-

TABLE 2. Percentage community particulate carbon fixation (% P), percentage community biomass (% B) and relative activity index (RAI, equal to % P/% B) of three phytoplankton species in high light, control, and phosphorus enriched continuous cultures.

Culture and date	*Synedra radians*			*Dinobryon sertularia*			*Ankistrodesmus falcatus*		
	% P	% B	RAI	% P	% B	RAI	% P	% B	RAI
+ Light									
Aug. 7		1.03			8.1			.44	
Aug. 8	.25		.24	11.5		1.42	.69		1.57
Aug. 9	.19			17.6			.63		
Aug. 11	.38			16.7			.76		
Aug. 13	.64			22.2			.74		
Aug. 17	.40		.36	20.3		1.00	.89		2.97
Aug. 18		1.12			20.4			.30	
Control									
Aug. 7		1.11			8.6			.63	
Aug. 8	.34		.31	11.3		1.31	.80		1.27
Aug. 9	.29			11.3			.52		
Aug. 11	.52			7.9			.62		
Aug. 13	.56			13.5			1.06		
Aug. 17	.46		.29	9.5		.49	1.18		3.19
Aug. 18		1.34			19.2			.37	
+P									
Aug. 7		1.16			9.1			.53	
Aug. 8	.32		.28	13.0		1.43	.57		1.08
Aug. 9	.31			7.3			.83		
Aug. 11	.66			7.5			.59		
Aug. 13	.96			10.5			.81		
Aug. 17	1.25		.47	15.4		.81	.80		2.16
Aug. 18		2.67			19.1			.37	

urable loss, extracellular release, which increased under phosphorus enrichment. Losses are a critical mediating link in the relationship between production and biomass.

EFFECT OF PHOSPHORUS ON *Dinobryon*

The results obtained with *Dinobryon sertularia* help resolve a conflict which exists concerning the nutrient requirements of the genus. Rodhe (1948) found that addition of 5–10 $\mu g \cdot L^{-1}$ phosphorus was toxic to *Dinobryon divergens* grown in filtered lakewater, whereas Lehman (1976) noted a linear phosphorus: final yield relationship to levels exceeding 100 $\mu g \cdot L^{-1}$ with several species grown on defined medium; he attributed Rodhe's results to possible potassium toxicity. The present study confirms that low level (10–20 $\mu g \cdot L^{-1}$) phosphorus addition does indeed inhibit production by *Dinobryon sertularia*. The rapid numerical decline noted (Fig. 3) and the increased extracellular release (Fig. 1) are both consistent with a direct loss through death in agreement with Rodhe's findings. Rapid numerical declines were not observed with either *Synedra* or *Ankistrodesmus* following inhibition of similar magnitude (Fig. 2, 4). Cells which survived the first few days either had a higher initial tolerance level or were capable of adaptation because the carbon fixation data point towards a higher final yield (Fig. 3), in support of Lehman's findings. Consequently, it appears that the effect phosphorus addition has on *Dinobryon* will depend strongly on the physiological state and makeup of the population: results indicating short-term toxicity need not be in conflict with a long-term increase in yield.

THE BIOASSAY APPROACH

The ability to monitor and predict the effects of environmental perturbations is of ever increasing importance. Long-term bioassays can be challenged on the grounds of bottle effects (cf. Vollenweider and Nauwerck 1961) while shorter incubations are suspect on the basis that they overly emphasize transient responses while completely neglecting longer-term effects. Raw lakewater continuous cultures provide one resolution to the problem by establishing an enclosed, manipulable system which will maintain realistic community composition for extended periods of time (deNoyelles et al. 1980).

Our study indicates that the detection of changes in biomass by individual species provides an accurate and direct means of quantifying perturbation effects when species responses are stable over several days as was the case with phosphorus stimulation of *Synedra radians* (Fig. 3). Track autoradiography provides the additional

sensitivity and temporal resolution necessary to monitor responses that are either consistently low level, as in the case of *Synedra radians* inhibition by light, or very variable as in *Dinobryon sertularia* where phosphorus enrichment changed from neutral to inhibitory to stimulatory in rapid succession.

The results clearly demonstrate the unsuitability of short-term bioassays for prediction of long-term perturbation effects. A bioassay lasting 24 h or less would have found particulate carbon fixation inhibited by phosphorus enrichment whereas the long-term effect was enhancement (Fig. 1). The inadequacy is compounded at the species level where a 2-h bioassay would indicate that phosphorus enrichment inhibited *Ankistrodesmus falcatus* (Fig. 4) and a 24-h bioassay would produce a similar result for *Dinobryon sertularia* (Fig. 3) whereas in fact neither species was inhibited in the long run. Similarly, the strong stimulation of *Synedra radians* by phosphorus was not observable during the first 24 h. It is thus clear that bioassays must last longer than 24 h so that initial shock effects can dissipate and adaptation or selection can occur, because it is the longer-term effect which provides the basis for community change in response to environmental perturbation.

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Physiological Indicators of Nutrient Deficiency in Lake Phytoplankton

F. P. HEALEY AND L. L. HENDZEL

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

HEALEY, F. P., AND L. L. HENDZEL. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Can. J. Fish. Aquat. Sci. 37: 442–453.

Physiological indicators of N and P deficiency were measured in summer populations of several central Canadian lakes. Measurements included six composition ratios (P/C, N/C, N/P, ATP/C, chlorophyll *a*/C, and protein/carbohydrate) and three metabolic indicators (alkaline phosphatase activity and P and N debts expressed per unit particulate ATP). Overall results show P deficiency is generally greater than N deficiency in all the small lakes studied, embracing a wide range of maximum chlorophyll concentrations and N/P loading ratios. In two larger, very turbid lakes, neither P nor N deficiency appeared important, and light limitation is suggested. In a series of lakes of known N and P loading rates, indicators of P deficiency increased but those of N deficiency showed little trend with increasing N/P loading ratio. Some indicators of P deficiency appeared to provide a sensitive means of following the movement of river water, and loss of P from it, through a large lake. Some comparisons of net phytoplankton with total seston showed surprisingly little interference from detritus in these measurements.

Key words: deficiency, nitrogen, phosphorus, physiological indicators, phytoplankton

HEALEY, F. P., AND L. L. HENDZEL. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Can. J. Fish. Aquat. Sci. 37: 442–453.

Nous avons mesuré, dans plusieurs lacs du centre du Canada, les indicateurs physiologiques de déficience en N et P. Parmi ces mesures, on a calculé six rapports de composition (P/C, N/C, N/P, ATP/C, chlorophylle *a*/C et protéines/hydrates de carbone) et mesuré trois indicateurs métaboliques (activité de l'alcaline phosphatase et déficits en P et en N exprimés en unités d'ATP particulaire). Les résultats généraux indiquent que la déficience en P est ordinairement plus grande que la déficience en N dans tous les petits lacs étudiés, englobant une gamme étendue de concentrations maximales de chlorophylle et des rapports de charge N/P. Dans deux lacs plus grands et très turbides, la déficience en P ou en N ne semble pas importante et nous croyons que la lumière est un facteur limitatif. Dans un ensemble de lacs à taux de charge de N et de P connus, les indicateurs de déficience en P augmentent, mais ceux de déficience en N montrent peu de tendance en fonction de l'augmentation du rapport de charge N/P. Certains indicateurs d'une déficience en P semblent assez sensibles pour suivre le mouvement de l'eau de rivière et la perte de phosphore qu'elle subit en passant dans un grand lac. Des comparaisons du phytoplancton tamisé avec le seston total montrent, chose surprenante, très peu d'interférence des détritus sur les mesures.

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SOME physiological properties of algae change with nutrient deficiency in ways characteristic enough for these changes to be used as indicators of the presence of particular nutrient deficiencies. Various aspects of the composition of natural populations of algae have been used to obtain information on nutrient limitation of the algae (Gerloff and Skoog 1954, 1957a, 1957b) and to follow changes in the growth of phytoplankton blooms (Antia et al. 1963). Increased cell surface alkaline phosphatase activity has also been suggested as a useful indicator of P deficiency (Kuenzler and Perras 1965; Fitzgerald and Nelson 1966) and increased ability to take up various nutrients as indicative of deficiencies in those nutrients (Fitzgerald 1968; Healey 1973a). These measurements have been applied as indicators of nutrient deficiency in natural populations in both marine (Haug et al. 1973; Jensen and Sakshaug 1973; Mague et al. 1977) and freshwater environments (Petersen et al. 1974; Megard and Smith 1974; Healey and Hendzel 1976).

The physiological basis of these indicators of nutrient deficiency has been reviewed (Healey 1973b, 1975). These reviews show that a large number of algae from diverse taxonomic and ecological groups respond to at least some nutrient deficiencies in similar ways. Furthermore, many of these responses are quantitatively similar enough that comparison with measurements on algae in culture can provide a means of interpreting measurements made on natural mixed populations. Several physiological indicators of N and P deficiency have been measured on plankton of some central Canadian lakes during the summers of 1974–77, to determine the relative extent of N and P deficiency in some quite diverse lake types. Based largely on evidence drawn from the physiological literature (Healey 1973b, 1975),

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the following measurements and interpretations were used. Low ratios of particulate P/C and high ratios of N/P are indicative of the presence of P deficiency. High alkaline phosphatase activity and high P debt (Healey 1973a) per unit biomass also show the presence of P deficiency. Because particulate ATP is a useful index of living biomass and because the ratio of ATP to biomass can be expected to drop as P deficiency increases (Holm-Hansen 1970; Bottomly and Stewart 1976; Cavari 1976), phosphatase activity and P debt per unit ATP are used here. Measurements per unit ATP can be expected to be more sensitive to P deficiency and less subject to detrital interference than composition ratios. Similarly, low N/C ratios and high N debt/ATP are interpreted as indicative of N deficiency. Ratios of protein/carbohydrate and chlorophyll/C fall in several algae with both P or N deficiency (Healey 1973b, 1975), and ATP/C has been reported to fall with N deficiency as well as P deficiency (Holm-Hansen 1970; Berland et al. 1970).

A brief summary of some of this work was presented by Healey (1978).

Materials and Methods

Study Area

Phytoplankton populations were studied in four areas in Manitoba and northwestern Ontario. In mid-July and again in mid-August 1974, surface samples were obtained from two stations in the South Basin of Lake Winnipeg. In June, August, and September three surveys were made in Southern Indian Lake, a large riverine lake in northern Manitoba (Hecky 1975). Surface samples were collected at six stations throughout this lake and in a nearby flooding reservoir, Notigi. On five occasions during June, July, and August 1976, surface samples were collected from seven lakes (two of which were divided and are treated separately) in the Experimental Lakes Area (ELA) of northwestern Ontario. Although originally oligotrophic, most of the lakes studied had been artificially enriched with various combinations of N, P, and C for a number of years and provided a range of trophic situations and a variety of known N/P loading ratios (Schindler and Fee 1974; Schindler 1977). On several occasions during the late spring and early summer of 1977, various lakes in the Ericksson–Elphinstone area of western Manitoba (Barica 1975) were sampled. Altogether the lakes covered a wide range of trophic states, from 2 to 400 μg chlorophyll *a*/L.

Sample Collection

Most samples were collected near the surface, usually by lowering large plastic bottles about 10 cm below the surface. When only net plankton was needed, this was collected by dragging a 10-μm mesh net through the upper 1 m of the water. Samples from hypolimnion blooms were collected by peristaltic pump. Samples were usually held near lake temperature for 2–10 h before completion of analysis; however, the Lake Winnipeg samples were delivered to the laboratory and analyzed between 20 and 30 h after collection. Where samples contained large concentrations of zooplankton, these were removed either by lighting from above to drive the zooplankton down and decanting or by size separation using meshes of different sizes. On some occasions net and nannoplankton were also separated using meshes of different sizes chosen to give the separation desired.

Procedures

Particulate C, N, and P samples were collected on Whatman GFC glass fibre filters (usually 47 mm diameter) preignited at 500°C and analyzed as in Stainton et al. (1974). Chlorophyll *a* samples were collected on GFC filters, extracted with methanol, and determined spectrophotometrically. Depending on the species composition of the phytoplankton, chlorophyll *a* in the presence of chlorophyll *b* was calculated by the formula of Holden (1965) or chlorophyll *a* in the presence of chlorophyll *c* by the formula of Jeffrey and Haxo (1968). Protein and carbohydrate samples were collected on preignited GFC filters. The filter was homogenized in distilled water and the suspension made to 5.0 mL. One-millilitre aliquots of this suspension were analyzed for protein by the Lowry procedure and for carbohydrate by the anthrone procedure both essentially as described in Herbert et al. (1971). ATP samples were collected on Sartorius cellulose nitrate filters (0.45 μm pore; 24 mm diameter) and extracted and analyzed as in Rudd and Hamilton (1973). Between collection and analysis, samples were stored frozen at −17°C for 2–4 wk.

Cell surface alkaline phosphatase activity was measured fluorometrically as in Healey and Hendzel (1979). Parallel determinations were made of total and soluble activities, the soluble activity being that passing through 0.45-μm Sartorius cellulose nitrate filters. The difference between these is reported here as particulate activity. P and N debts were measured by adding a final concentration of 5 or 10 μM KH_2PO_4 or NH_4Cl to 100 mL of whole lake water and leaving this in darkness near lake temperature for 24 h. Triplicate 10-mL samples were removed at the beginning and end and analyzed for soluble reactive phosphorus or ammonium (Stainton et al. 1974). The nutrient debts were calculated as the nutrient removed over a 24-h period per unit of biomass (Healey 1973a).

Light penetration into the lakes was measured with a quantum meter (Lambda Instr. Co., model LI-185).

Results and Discussion

During the past several years, measurements of physiological indicators of N and P deficiency have been made on summer phytoplankton populations in several lakes to determine their usefulness in gaining information about the lakes. In this discussion we will first compare the levels of deficiency found in each of the lakes or lake groups, and then examine the application of these measurements to some more special situations.

Comparison of Physiological Indicators in Several Lakes

Division points between presence and absence of deficiency — Figure 1 compares several physiological indicators of N and P deficiency in the five lakes or lake groups studied. It allows comparison both among

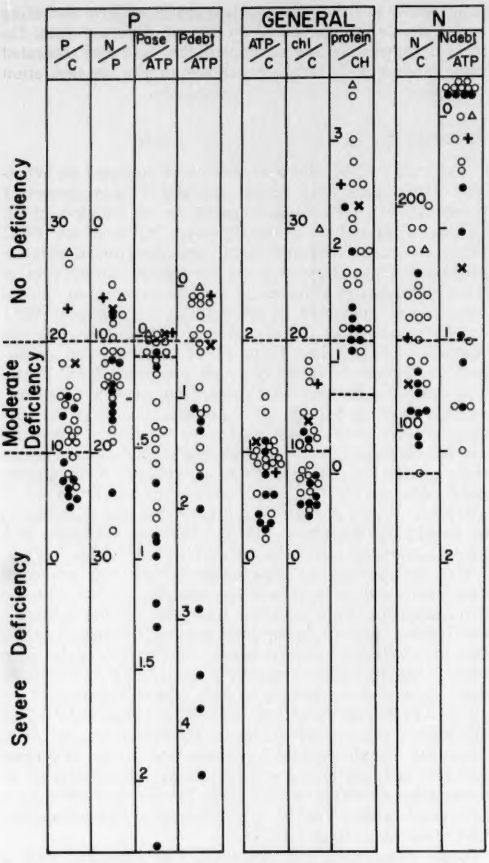

FIG. 1. Average summer epilimnion values for various physiological indicators of N and P deficiency in several central Canadian lakes. P and N headings indicate parameters indicative of P and N deficiency, respectively; general indicators cannot on the basis of present evidence be ascribed with any certainty to either N or P deficiency. The values of all these parameters taken as division points between severe, moderate, and no deficiency (horizontal lines) are based on measurements on algal cultures (Healey 1975; Healey and Hendzel 1979) as discussed in the text. Data points are: average of 4 or 5 summer epilimnion samplings in each of 9 ELA lake basins, ●; average of 2 midsummer samplings in each of 16 small prairie lakes (Erickson district), ○; average of early, mid, and late summer samplings at 6 stations distributed through Southern Indian Lake, ×; average of early, mid, and late summer sampling of Notigi Reservoir (east basin), +; and average of 4 midsummer samplings from the South Basin of Lake Winnipeg, △. Composition ratios are μg particulate, P, N, ATP, or chlorophyll *a* (chl)/mg particulate C; μg particulate N/μg particulate P; and μg protein/μg carbohydrate (CH). Nutrient debts are μmoles ammonium or phosphate removed in 24 h darkness/μg particulate ATP. Alkaline phosphatase activity (Pase) is μmole *o*-methylfluorescein phosphate hydrolyzed·h^{-1}·μg particulate ATP^{-1}. The group of data points at the top of the N debt column indicate negative debts (net release of ammonium). No attempt has been made to show the distribution of their actual values.

the lakes and between the characteristics of lake populations and the approximate division points between the presence and absence of P and N deficiency based on measurements on laboratory cultures. With the exception of ATP/C and alkaline phosphatase activity, the values shown in Fig. 1 as division points between the presence of severe, moderate and no N or P deficiency are taken from Healey (1975). In that publication, values were given mostly per unit organic dry weight because that is the way most were reported in the literature surveyed. Division points in Fig. 1 for P/C, N/C, and chlorophyll *a*/C were obtained from the corresponding ratios per unit organic dry weight by considering organic C as half the organic dry weight (Healey 1975). On the basis of rather limited evidence (Holm-Hansen 1970; Cavari 1976; Bottomley and Stewart 1976), values of 2 μg ATP/mg organic C or less are associated with P or N deficiency. On the same evidence, P or N deficient algae can be expected to have ATP at 1 μg/mg organic dry weight or less, making the division points for P or N debts per μg ATP the same as those per milligram organic dry weight. Alkaline phosphatase activities indicative of P deficiency using *o*-methylfluorescein phosphate as substrate (as done here) are much lower than those measured with *p*-nitrophenyl phosphate, summarized by Healey (1975). The division points for phosphatase activity are those found by Healey and Hendzel (1979) using *o*-methylfluorescein phosphate.

Lakes primarily P-limited — On the basis of both composition and metabolism, the summer populations of all the ELA lakes studied showed clear signs of P deficiency, generally rather severe deficiency. Some indication of N deficiency was also present, but this was less severe than P deficiency. Ratios of N/C generally indicated moderate N deficiency while N debts lay mostly in the range expected for N-sufficient algae. The lakes shown included both unmanipulated oligotrophic basins and those receiving various N, P, and C enrichments. They covered a wide range of maximum chlorophyll *a* concentrations, from 2 to 60 μg/L. The spread in several of the parameters shown is explained below, at least in part, on the basis of the range in N/P loading ratios found in these lakes.

In spite of great differences in underlying geology and the more advanced eutrophication found in many of them, the degree of deficiency reached by summer populations in the Erickson lakes was remarkably similar to that of summer populations in the ELA lakes. The distribution of all the parameters measured overlapped extensively. However, P/C ratios and more par-

TABLE 1. Comparison of light penetration in three Erickson lakes (average of three mid-summer values) and Southern Indian Lake (average of June, August, and September values).

Lake	Mean depth (m)	1% light depth (m)	1% light depth / mean depth
Erickson 255	1.7	3.2	1.9
318	1.4	2.1	1.5
885	1.9	1.7	0.90
Southern Indian Lake, basin 1	8.5	4.5	0.47
2	8.1	4.8	0.59
4	12.7	6.3	0.50
6	4.0	5.6	1.4

ticularly phosphatase activities and P debts showed the degree of P deficiency reached in the prairie lakes was slightly less, on average, than in the ELA lakes. The N/C ratios suggested slightly less N deficiency also. The values reported here for these lakes are comparable to those found previously in some of the same lakes (Healey and Hendzel 1976).

While present evidence does not allow interpretation of changes in ATP/C, chlorophyll *a*/C, or protein/carbohydrate ratios as indicative of specific nutrient deficiencies (Healey 1973b, 1975; Holm-Hansen 1970), the distribution in Fig. 1 suggests the ATP and chlorophyll *a* contents of both ELA and Erickson seston were depressed by P deficiency, while the protein/carbohydrate ratio followed the lesser N deficiency. The latter is surprising in view of the depressed protein/carbohydrate ratio seen in many P deficient algae in culture (Healey 1975). The ATP/C (and perhaps chlorophyll *a*/C) ratios could also be depressed by inclusion of detrital C. Evidence for this effect on ATP/C ratios is given below in comparison of the composition of net plankton with total seston samples.

Lakes not primarily P-limited — Turning to the larger lakes, a quite different picture emerges. The midsummer phytoplankton of Lake Winnipeg appeared to be deficient in neither P nor N. Ratios of P/C, N/P, N/C, chlorophyll *a*/C and protein/carbohydrate were in the range of algae cultured in the presence of adequate available P and N, while phosphatase activity and P and N debts were undetectable. The nutritional status of the phytoplankton of Southern Indian Lake was similar to that of Lake Winnipeg in several respects. Composition ratios indicated the presence of only moderate P or N deficiencies at most, while phosphatase and P and N debts, less affected by detritus than the composition ratios, showed P and N deficiency to be slight or absent. While most indicators showed Southern Indian Lake plankton to be somewhat more N and P deficient than that of Lake Winnipeg, neither seem primarily nutrient limited. Other evidence suggests they may be primarily light-limited. Both are large (2000–3000 km^2), relatively shallow (10 m mean depth) lakes which do not stratify during the summer. The water is turbid due to sediments brought in by rivers and resuspended from the bottom. As a result, the phytoplankton probably spend much of the time below the photic zone and this could result in light limitation.

Comparison of the results from Southern Indian Lake with those from the Erickson lakes suggests that the largely inorganic (clay particle) turbidity of the former limited light availability to the phytoplankton more than did the dense blooms of the latter. Comparison of light penetration data for the two lake types supports this and shows the major factor was the different depths of the lakes (Table 1). Both were mixed to the bottom. While the 1% light penetration level in Southern Indian Lake during the summer averaged about twice that in the Erickson lakes, the mean depths of the Southern Indian Lake basins generally exceeded those of the Erickson lakes by a greater factor. Thus the average light intensity that the Southern Indian Lake phytoplankton was exposed to was less than that in the Erickson lakes.

The phytoplankton of Notigi Reservoir appeared for the most part less P- and N-deficient than that of Southern Indian Lake. Because the morphometry of Notigi is very different from that of Southern Indian Lake, the same explanation cannot hold. However, the reservoir was in the process of filling and new areas were being flooded while these measurements were being made. Nutrient input from the newly flooded areas may have kept the plankton in a nutrient-sufficient state.

Persistence of P-limitation in hypereutrophic lakes — In both the ELA and the Erickson lakes little relationship was found between the maximum chlorophyll *a* concentration and the degree of P or N deficiency. At the lower chlorophyll *a* concentrations, a wide range of values is found, indicating that some variable other than chlorophyll *a* concentration is important in determining P and N deficiency (Fig. 2). At higher chlorophyll *a* concentrations, indications of P deficiency are lessened, but those of N deficiency are not increased. Thus, in the region of 100–400 μg chlorophyll *a*/L, P deficiency exceeded N deficiency and dis-

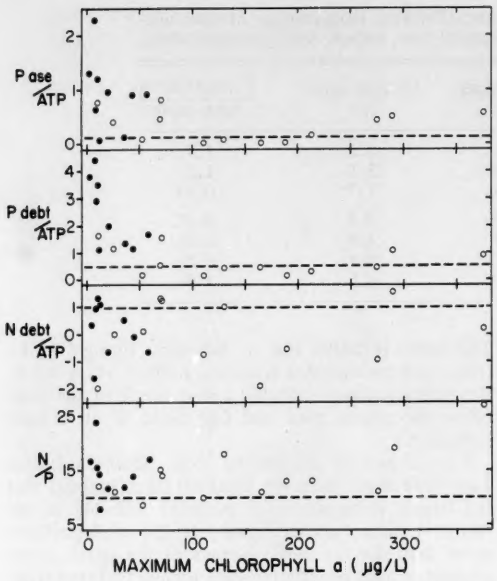

FIG. 2. Variations in some indicators of N and P deficiency with chlorophyll *a* concentration in ELA (●) and Erickson (○) lakes. Based on measurements on algal cultures (Healey 1975; Healey and Hendzel 1979), values above the dotted lines in the phosphatase, P debt, and N/P panels indicate the presence of P deficiency and above that in the N debt panel, N deficiency. Units as in Fig. 1.

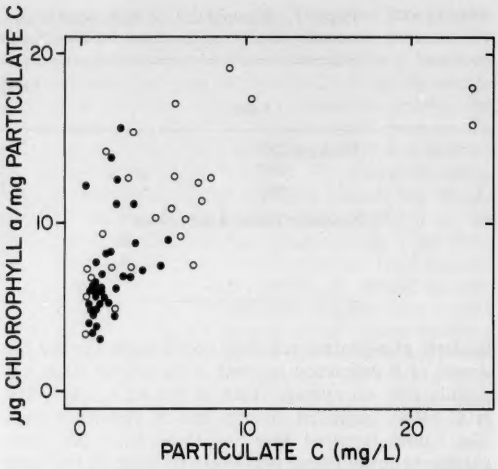

FIG. 3. Variations of the chlorophyll *a*/C ratio with concentration of total sestonic C in ELA (●) and Erickson (○) lakes.

tinct evidence of P deficiency is still found, although lessened, probably by the onset of light limitation. Further evidence for acclimation to decreased light in the lakes containing higher seston concentrations can be seen in the tendency for chlorophyll *a*/C to increase with increasing total organic seston (particulate C) concentration (Fig. 3). As well as varying with nutrient deficiency, increased chlorophyll per unit biomass is a common response to decreased light intensity (Healey 1975).

The suggested tendency for N deficiency to replace P deficiency with increasing degree of eutrophication, as reflected by increased chlorophyll *a* concentration (Forsberg 1975; Forsberg et al. 1975), was not seen in these lakes (Fig. 2). Although the degree of P deficiency decreased, that of N did not increase. This is not surprising in view of the fact that the densest blooms we studied were dominated by N_2-fixing blue-green algae, species of *Aphanizomenon* and *Anabaena*. While P deficiency exceeded N deficiency in the Erickson lakes at most sampling times, on a few occasions the N debt considerably exceeded the P debt. The four occasions when the N debt exceeded the P debt by more than twofold are shown in Table 2a. On each occasion the N/C and the protein/carbohydrate ratios

TABLE 2. Phytoplankton characteristics on occasions when N debt exceeded P debt by more than twofold (a) and the same measurements 2 wk earlier or later (b). Lakes in the Erickson area sampled during the summer of 1977. For further information on these lakes, see Barica (1975). Units as in Fig. 1.

	Lake	Date	P/C	N/P	Phosphatase activity	P debt	ATP/C	Chlorophyll *a*/C	Protein / Carbohydrate	N/C	N debt
a)	882	July 6	17	8.8	0.013	0.11	1.07	12.44	1.6	150	0.53
		July 8	11	13.6	0.027	0.27	0.65	12.78	2.0	150	1.65
	302	July 8	13	11.5	0.44	0.33	0.85	10.17	1.3	140	2.11
	303	July 20	16	10.6	0.025	0.059	1.22	18.14	2.3	170	0.25
b)	882	July 19	6	31	0.16	0.88	0.21	15.34	2.3	180	0.82
	302	July 19	10	16.6	0.55	0.65	1.00	12.53	1.2	170	0.88
		July 21	10	16.4	0.36	0.58	1.24	10.83	1.4	160	0.84
	303	July 8	9.0	19.3	0.20	0.29	0.81	15.92	2.9	180	0.21

were high enough to indicate the absence of N deficiency, contrasting with the two high N debts seen. The P/C ratios indicated moderate P deficiency, while the N/P ratios showed only slight or no P deficiency. On three of the four occasions the phosphatase activity was low relative to levels expected in P deficient algae, and the four P debts indicated no P deficiency. Except for the two higher N debts, the general picture given in Table 2a is one of slight N or P deficiency. Although these measurements were made on dense blooms, measurements at as high or higher chlorophyll *a* concentrations on other lakes showed clear signs of well-developed P deficiency. Furthermore, this situation did not persist long. While the chlorophyll *a* concentration remained similar, Lakes 882 and 302 were clearly more P than N deficient 2 wk after, and Lake 303 two weeks before, the dates when N debt exceeded P debt (Table 2b). Thus it is unlikely they had reached light limitation. Comparison of parts a and b of Table 2 shows that the changes which occurred to give the high ratios of N debt to P debt in Table 2a involved more a decrease in P deficiency (rise in P/C and sharp drop in phosphatase activity and P debt) than an increase in N deficiency (N/C fell and N debt rose by a lower factor than did the corresponding P parameters). Thus, these situations where N debt exceeded P debt may reflect transient situations in which P input exceeded N input. They do not give evidence that these blooms were more N than P deficient. However, they do emphasize the importance of sampling at more than one time through the growth of a bloom and measuring more than one indicator of deficiency.

Variation in P and N Deficiency with Variation in N/P Loading Ratio

Variations in epilimnion populations — As part of an experimental eutrophication program, the P and N loading to several ELA lakes has been monitored. As a result a series of lakes with a variety of known N/P loading ratios was available. Physiological indicators of P and N deficiency were measured on epilimnion samples of nine lake basins at five times through the summer of 1976 to test the ability of these indicators to reflect the different loading ratios. Average values for each basin are shown as a function of the N/P loading ratio in Fig. 4. These results illustrate increasing P deficiency (increasing phosphatase activity, P debt, and particulate N/P ratio and decreasing P/C and ATP/C ratios) with increasing N/P loading ratio, especially at the lower loading ratios. Only the phosphatase activities and particulate N/P ratios of the lakes with the lowest N/P loading ratios were suggestive of no P deficiency. All other values indicated the presence of P deficiency, becoming more severe as the N/P loading ratio rose. To a lesser extent, N deficiency tended to decrease with increasing loading ratio. However, the tendency of N/C and protein/carbohydrate to rise and of N debt to fall with increasing N/P loading ratio is much weaker than the trends in indicators of P deficiency. The increase in protein/carbohydrate ratio as P deficiency became more severe is a further indication that this ratio reflects N rather than P deficiency. Particulate N/C values were all in the range of moderate N deficiency. Only those lakes with the lowest N/P loading ratios had N debts high enough and protein/carbohydrate ratios low enough to be indicative of moderate N deficiency. At higher N/P loading ratios, the latter measurements indicated no N deficiency. The chlorophyll *a*/C ratio (not shown) showed no trend with loading ratio. The overall result of changing the N/P loading ratio of these initially oligotrophic lakes is to change the degree of P deficiency, which was already severe in the untreated basins (239 and 302S).

Even at the lower N/P loading ratios, N deficiency did not replace P deficiency, probably because N_2 fixation and preferential regeneration of N from sinking particles and from sediments (Schindler 1976) occurred at sufficient rates to raise the effective N/P loading ratio above that shown in Fig. 4. Planktonic N_2 fixers (*Anabaena* spp.) were common in Lakes 226 NE and 227. While these were not evident in Lake 261, N_2 fixation in the periphyton has previously been reported (Schindler 1977). In Lake 261, both N and P deficiencies were equally, but only marginally, developed. This suggests that N_2 fixation in this lake was not as effective in overcoming the low N/P loading ratio as it was in the other lakes.

There were no seasonal trends which were consistent between lakes. While large fluctuations occurred through the summer in some indicators in some lakes, most maintained similar values through the sampling period.

The results in Fig. 4 emphasize that the degree of P deficiency found in a lake is not dependent of the rate of P loading alone, but on the ratio of P loading to the rate of supply of N and other potentially limiting factors. For instance, Lake 304 with the highest P loading rate had higher phosphatase activity than Lakes 261 and 227 with much lower P loading rates, but a similar activity to 303 which had a P loading rate similar to lakes with much lower phosphatase activities. None of the indicators showed any trend with P loading alone.

Artificial loading of N, P, and C to Lake 302 N was to the hypolimnion. This had little effect on the size of epilimnion populations (Schindler and Fee 1974). The total loading to this basin gave an N/P ratio of 6.2 in 1976 (shown in Fig. 4 as an open circle). For most indicators (measured on epilimnion populations) this point lay well off the relationship found in the other lakes. However, when the same data were plotted at the natural N/P loading ratio for the basin (33), close agreement with the other lakes was obtained. Thus, in lakes of this sort (with recent eutrophication and a stable summer stratification) both the size of the summer population and its degree of P deficiency

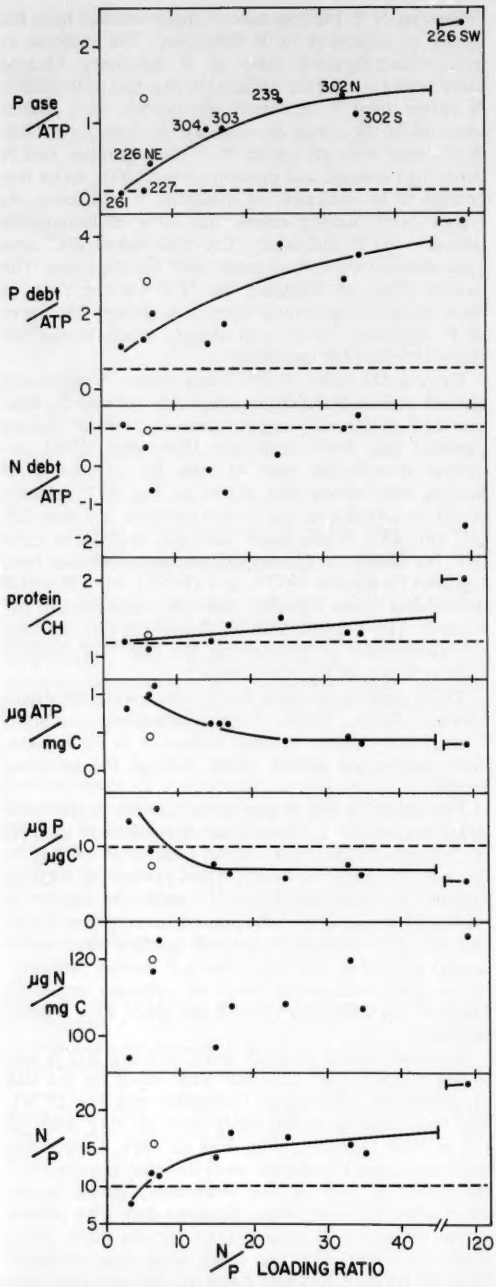

FIG. 4. Variation of several indicators of N or P deficiency in epilimnion phytoplankton with N/P (by weight) loading ratio in ELA lakes. Data points in the lower panels can be identified as to lake number by referring to the same loading ratio in the top panel. Lake 302 N is plotted opposite its natural loading ratio. The open circles refer to the total loading (artificial hypolimnion as well as natural surface loading) ratio to this basin. These loading ratios do not include N_2 fixation, which would raise the lowest ratios (Schindler 1977). Based on measurements on algal cultures (Healey 1975; Healey and Hendzel 1979), values above the dotted lines in the phosphatase, P debt, and N/P panels indicate the presence of P deficiency; above that in the N debt panel, N deficiency; below that in the P/C panel, severe P deficiency; and below that in the protein/CH panel, probably N deficiency. All ATP/C values indicate P deficiency and all N/C, moderate N deficiency. Units as in Fig. 1.

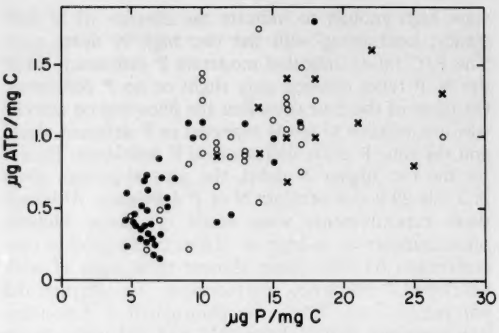

FIG. 5. Relationship between particulate ATP/C and particulate P/C in several ELA (●) and Erickson (○) lakes and in Southern Indian Lake (×).

reflect natural surface loading much more than they do hypolimnion loading.

Figure 4 shows that both the P/C and ATP/C ratios, when averaged over the summer for each lake basin, decreased with increasing N/P loading ratio in similar manners. This suggests that the ATP content of phytoplankton may decrease as its P content does. To evaluate this further, ATP/C ratios were plotted as a function of P/C ratios using all the epilimnion total plankton samples collected during this study. Figure 5 shows a tendency for the ATP/C ratio to increase with the P/C ratio, moving from the ELA lakes with the lowest P/C ratios to the Erickson lakes with generally higher ones. In the Southern Indian Lake samples, with the highest P/C ratios, this relationship is lost. These results support other evidence from both field (Cavari 1976) and laboratory (Holm-Hansen 1970; Bottomly and Stewart 1976) studies that an important factor affecting the ATP/C ratio of plankton samples is the degree of P deficiency, and casts doubt on the very constant ATP/C ratios recently reported for natural populations (Paerl and Williams 1976). That this ratio can also be greatly affected by the detrital content of the sample is illustrated in the final section.

TABLE 3. Comparison of summer average nutrient deficiency symptoms of hypolimnion and epilimnion populations in Lake 302 (ELA). The North Basin received hypolimnion loading of C, N, and P. Units as in Fig. 1.

	South Basin		North Basin	
	Epilimnion	Hypolimnion	Epilimnion	Hypolimnion
P/C	6.1	9.4	7.5	24
N/P	14.4	13.4	15.5	6.1
Phosphatase activity				
pH 8.5	1.2	0.35	0.66	0.32
pH 5.5	—	3.8	—	1.1
P debt	3.5	1.53	2.9	1.2
ATP/C	0.38	0.71	0.45	0.53
Chlorophyll *a*/C	4.5	12.5	5.3	26.0
Protein/carbohydrate	1.3	2.4	1.3	2.3
N/C	107	121	120	140
N debt	1.3	−0.4	0.96	0.0

Characteristics of hypolimnion populations — Besides measurements on epilimnion populations, measurements were also made on hypolimnion populations in four basins, often including hypolimnion blooms of the sort described by Fee (1976). Like the epilimnion populations, those in the hypolimnion were more P than N deficient (Table 3). However, they were distinctly less P and N deficient than the epilimnion populations, presumably because they were more light limited. P and N deficiency was further decreased by hypolimnion loading of these nutrients to the North Basin of Lake 302. While epilimnion populations showed greater alkaline than acid phosphatase activity, the reverse was true for the hypolimnion populations (Healey and Hendzel 1979). Thus it is difficult to compare phosphatase activities between them. Hypolimnion populations had higher acid phosphatase than epilimnion had alkaline. However, an alga isolated from a hypolimnion bloom, *Synura uvella*, when grown into severe P deficiency had higher acid phosphatase activity per unit dry weight than other algae had alkaline phosphatase activity at similar degrees of P deficiency (Healey and Hendzel 1979). Thus, the fact that acid phosphatase activity in the hypolimnion was greater than alkaline phosphatase activity in the epilimnion cannot be interpreted as indicating greater P deficiency in the hypolimnion, especially when the other indicators showed the opposite.

Spacial Variations in P Deficiency Through a Large Lake

On three occasions during the summer of 1975, samples were collected at six stations over the surface of Southern Indian Lake. This provided an opportunity to look at the distribution of P deficiency through this large lake, particularly in relation to how this was affected by the flow of river water through the lake. At that time, prior to diversion in a hydroelectric project, the river flow entered the lake in the southwest and moved through to exit in the northeast (Fig. 6). During midsummer, both the phosphatase activity and the P debt of the net phytoplankton were low near the inflow and had increased only slightly by station 3A. Ratios of P/C and ATP/C were relatively high. In the North Basin of the lake (station 4B), phosphatase activity and P debt rose while P/C and ATP/C ratios of the net plankton fell, indicating increased P deficiency as the river water moved through the lake. Moving laterally away from the main flow of the river, P deficiency increased more markedly. Station 2A is located in a bay separated from the main body of the lake by an island and was characterized by elevated phosphatase activity and P debt and by a depressed P/C ratio. Station 6C, located in a bay isolated from the main prediversion flow of the river, showed the highest P deficiency found in the lake. Previous studies (Hecky 1975) suggest that there is a distinct plume of river water passing stations 1A, 2E, 3A, and 4B, and that the bay containing station 6C has a much larger residence time and lower P loading rate than the main body of the lake. Besides being isolated from the prediversion flow of the river, this station had the most favorable light conditions (Table 1). While this pattern was evident during the midsummer, it was not seen in early or late summer, when the influence of the river may have been masked by greater wind-driven mixing of the lake. At these times, P deficiency was minimal at all stations (at the levels of inflow stations in Fig. 6) suggesting that P was being added by sediments at all stations. However, during midsummer these measurements seemed to provide a sensitive indicator of the flow of river water through the lake and the removal of P from it.

Comparison of Net and Nannoplankton

During the replacement of spring populations

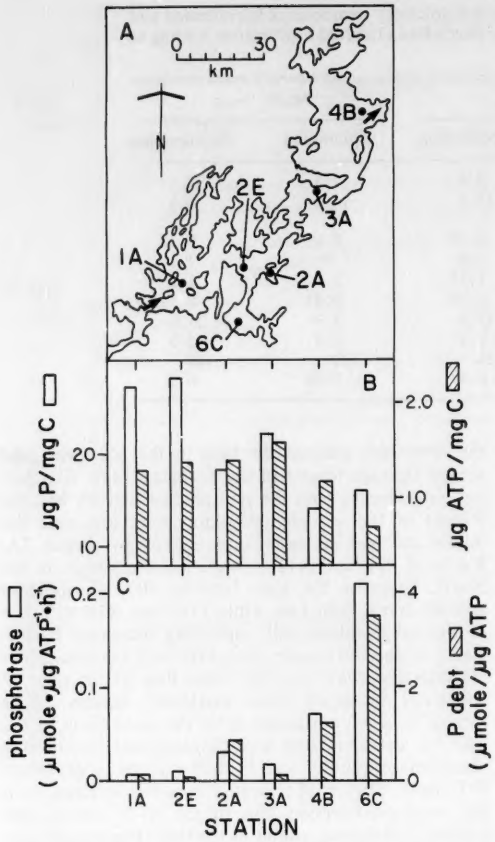

FIG. 6. Variation in some indicators of P deficiency measured on net phytoplankton through Southern Indian Lake in August 1975. A, Location of sampling stations. B, P/C and ATP/C ratios of net phytoplankton. C, Alkaline phosphatase activity and P debt of net phytoplankton

(dominated by nannoplankton) by populations of potentially N_2-fixing net plankton (species of *Aphanizomenon* and *Anabaena*) in four basins in the Erickson area, these were separated by size to determine what effect the ability to fix N_2 had on the relative P and N deficiencies of the two populations. Representative results are shown in Table 4. The two fractions did not differ appreciably in degree of P deficiency but the net plankton was richer in N (higher N/C and N/P ratios and lower N debt) than the nannoplankton. The nannoplankton had a lower ATP/C ratio, probably reflecting its greater detrital contamination.

During midsummer sampling at Southern Indian Lake, net and total plankton samples were collected at each of five stations through the lake. Using various size meshes the net plankton sample was freed as much as possible of detritus and zooplankton. Characteristics of the two types of samples (Table 5) were compared to determine the effect of detritus on indicators of nutrient deficiency. While characteristics from individual stations often differed between the two types of samples, the same general picture of slight N or P deficiency for the lake as a whole emerges from the average characteristics of either. As above, the ATP/C ratio of the total plankton was less than that of the net plankton, again probably reflecting the greater detrital content of the former.

Conclusion

The various physiological indicators of N and P deficiency used here have considerable experimental support from laboratory studies (Healey 1973b). We have shown here that several of these change in a consistent way with N/P loading ratio over a wide range of this ratio and chlorophyll *a* concentrations. This provides evidence from a field situation that they are reliable indicators of the degree of N and P deficiency and also suggests that they may be useful in estimating N/P loading ratios of lakes, a ratio that seems important in determining the occurrence of blooms of blue-green N_2 fixers (Schindler and Fee 1974).

The physiological parameters measured here pre-

TABLE 4. Characteristics of net and nannoplankton in Lake 318 (Erickson). Average of six determinations during May and June 1977 when a net plankton population dominated by first *Anabaena* and then *Aphanizomenon* coexisted with a nannoplankton population dominated by small green algae and diatoms.

	μg/mg C				wt/wt		Activity/ATP		
	P	N	Chl	ATP	N/P	Protein / Carbohydrate	P ase[a]	P debt[b]	N debt[b]
Net plankton	17	190	9.1	2.0	11.3	2.99	0.15	0.38	−1.6
Nannoplankton	18	150	8.5	1.1	8.3	2.85	0.19	0.50	−0.10

[a]Alkaline phosphatase activity as μmole *o*-methylfluorescein phosphate hydrolyzed·μg $ATP^{-1}\cdot h^{-1}$.
[b]μmole phosphate or ammonium taken up/μg ATP; negative N debts indicate net release of ammonium during incubation.

TABLE 5. Average characteristics of net phytoplankton and total seston at five stations through Southern Indian Lake during August 1975. At each station, total seston and net phytoplankton (10-μm mesh, further purified of zooplankton as in methods) were collected simultaneously. Comparison not made with N and P debts.

	μg/mg C						
	P	N	Chl	ATP	N/P	Protein / Carbohydrate	Alkaline phosphatase activity[a]
Net plankton	19.4	126	13.4	1.23	6.6	2.4	0.066
Total seston	22.8	118	14.2	0.79	5.7	2.4	0.095

[a]Alkaline phosphatase activity as μmole *o*-methylfluorescein phosphate hydrolyzed·μg $ATP^{-1}\cdot h^{-1}$.

sumably reflect nutrient supply during some time period preceding sampling. In situations when nutrient input to the euphotic zone is changing with time, single measurements of physiological indicators may reflect the nutrient situation over only a short period, and not the nutrient limitation the summer population may eventually achieve. This may account for the changes seen in the Erickson lakes (Table 2). However, measurements at several sampling times through the period of maximum summer chlorophyll should reflect the controlling nutrient limitation of the lake. This is supported by the relationship of the indicators to the N/P loading ratio in the ELA lakes and the fact that both whole lake enrichments (Schindler and Fee 1974) and the measurements reported here show these lakes to be primarily P-limited.

The application to natural populations of phytoplankton of methods devised with pure cultures of algae can be criticized on the basis of interference from bacteria and nonliving seston in the phytoplankton samples. That this does not seem to be a major problem is shown by the similarity of average characteristics of total seston and net plankton found in Southern Indian Lake. Furthermore, detrital interference should be minimal in P debt, N debt, and alkaline phosphatase activity expressed per unit of particulate ATP.

The characteristics of P and N deficiency in the summer phytoplankton populations of the lakes studied here showed that, when N or P deficiency was present, P deficiency generally exceeded that of N. This was true over a broad range of trophic states (as indicated by maximum summer chlorophyll *a* concentrations) and N/P loading ratios. This supports the accumulating evidence for the generality of P limitation in lakes, which has otherwise been based largely on the correlation between maximum summer chlorophyll and maximum total P (Sakamoto 1966; Dillon and Rigler 1974) and whole-lake enrichment experiments (Schindler and Fee 1974). However, it does not support recent suggestions (Forsberg 1975; Forsberg et al. 1975) that with advanced eutrophication N replaces P limitation. This suggestion was based on small-scale enrichment experiments in which the ability of nutrient additions to increase the growth yield of a test alga in filtered lake water was measured. Such small scale enrichments can give misleading results because both other potential limiting factors (such as light or grazing) and natural loading and cycling of nutrients are altered or eliminated.

Phytoplankton in the most eutrophic lakes, where N limitation has been suggested, may have been light limited and the apparent N limitation revealed in these tests may have been potential rather than real. For example, Coveney et al. (1977) describe a bloom in which SRP was always present in excess of plant needs (300 μg/L or more) while inorganic N dropped to quite low but still detectable levels (30 μg/L). A nutrient enrichment experiment at this time would undoubtedly have shown an apparent N limitation. However, the particulate N/P ratio (which must have been dominated by the algal bloom) was abut 10, indicative of little or no N deficiency (Healey 1975) and dissolved inorganic N seemed never to be driven to undetectable levels. Thus actual N deficiency was very unlikely. In this bloom maximum biomass may have been limited by some factor other than P or N. However, it should be reemphasized that in the densest blooms we studied, there was little indication of light limitation but a clear indication of P deficiency even at chlorophyll concentrations approaching 400 μg/L. This was probably due to the fact that the lakes where these blooms occurred were very shallow. In larger, deeper, unstratified lakes, largely inorganic turbidity appears to have resulted in light limitation.

Measurement of the various physiological indicators of nutrient deficiency discussed here provides a means of determining whether P or N deficiency is important in phytoplankton populations and what the relative importance of these two deficiencies are. This has application in limnological studies as the variation of P or N deficiency in populations may be important in the growth and collapse of blooms (Healey and Hendzel 1976). While we found little difference between nannoplankton and potentially N_2-fixing net plankton during early summer replacement of the former by the latter in two prairie lakes, others have seen the ability to fix N_2 reflected in decreased N deficiency and increased P deficiency (Mague et al. 1977). It is possible that N_2 fixation was not occurring at the time of our study and may not have been important in the replace-

ment of non N_2-fixers by potential N_2-fixers here. Indeed, both N and P deficiency seemed minimal in both populations. The distribution of P deficiency in Southern Indian Lake shows the potential of such measurements as phosphatase activity for tracing the movement of P through a lake. Accepting P as the nutrient most likely to be limiting in lakes and certainly the one most amenable to management (Schindler 1977), the measurement of physiological indicators would be helpful in confirming whether blooms in a particular lake were limited by P or by some other nonnutrient factor before control of P was undertaken. They could also be used to monitor the effectiveness of initial steps in P control as reflected in increased P deficiency in the blooms before a decrease in biomass is seen. For these applications, the routine measurement of a few of the most sensitive used here (such as the N/P ratio of total seston or net phytoplankton or alkaline phosphatase activity, P debt, or N debt per unit of particulate ATP) would be useful.

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Whole-Lake Radiocarbon Experiment in an Oligotrophic Lake at the Experimental Lakes Area, Northwestern Ontario

R. H. HESSLEIN,[1] W. S. BROECKER, AND P. D. QUAY

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York, NY 10964, USA

AND D. W. SCHINDLER

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

HESSLEIN, R. H., W. S. BROECKER, P. O. QUAY, AND D. W. SCHINDLER. 1980. Whole-lake radiocarbon experiment in an oligotrophic lake at the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 37: 454–463.

To gain more insight into the nature of carbon cycling in lakes and to provide a check on estimates of carbon fluxes obtained by more conventional means, 1 Ci (= 37 GBq) of C^{14} as $NaHCO_3$ was added to the epilimnion of Lake 224, a dimictic, oligotrophic lake of the Canadian Shield near Kenora, Ontario. The dominant loss from the dissolved inorganic carbon (DIC) pool was via $C^{14}O_2$ evasion to the overlying atmosphere. The next most important loss from the DIC pool was by photosynthetic fixation of inorganic carbon by epilimnetic phytoplankton. About half of the C^{14} thus incorporated into the particulate organic carbon (POC) pool was converted into soluble organic molecules which became part of the epilimnetic dissolved organic carbon-14 (DOC) pool. Since the amount of C^{14} lost to the sediments of the epilimnion, to the hypolimnion, and to periphyton biomass was not significant to the C^{14} mass balance over the duration of the experiment, the rate of gas exchange can be calculated by measuring the decrease in epilimnetic C^{14} inventory ($DIC^{14} + POC^{14} + DOC^{14}$) over a specific time period. Using the stagnant boundary model and pCO_2 values calculated from pH, temperature and DIC data a range of stagnant film thicknesses of 212–316 μm was obtained. To provide a check on the film thickness calculated from C^{14} inventories 10 mCi if Ra^{226} was also added to the epilimnion of L224. Measurements of Rn^{222}, the gaseous daughter product of Ra^{226}, allowed an independent estimate of the film thickness. The average value of 200 μm obtained in this way is consistent with that obtained for $C^{14}O_2$ evasion. A simplified model was also constructed to describe the behavior of the POC and DOC pools. This model produced results in excellent agreement with the photosynthetic rate averaging 65 mg $C \cdot m^{-1} \cdot d^{-1}$ measured using C^{14} and the Fee incubator technique. The model also suggests that only about 10% of the POC + DOC pool is active in the photosynthetic process on the time scale of 30 d.

Key words: whole-lake radiocarbon experiment, gas exchange, primary production, radium^{226}, radon^{222}, carbon^{14}, carbon in lakes

HESSLEIN, R. H., W. S. BROECKER, P. D. QUAY, AND D. W. SCHINDLER. 1980. Whole-lake radiocarbon experiment in an oligotrophic lake at the Experimental Lake Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 37: 454–463.

Afin de mieux comprendre le cycle du carbone dans les lacs et de vérifier les estimations des flux de carbone obtenues par des moyens plus conventionnels, on a ajouté 1 Ci (= 37 GBq) de C^{14} comme $NaHCO_3$ à l'épilimnion du lac 224, un lac oligotrophe dimictique du Bouclier canadien près de Kenora, en Ontario. Le carbone inorganique dissous (DIC) est perdu surtout via la fuite de $C^{14}O_2$ dans l'atmosphère sus-jacent. Une autre perte, seconde en importance, du pool de DIC résulte de la fixation photosynthétique de carbone inorganique par le phytoplancton épilimnétique. Environ la moitié du C^{14} incorporé de cette façon dans le pool de carbone organique particulaire (POC) se convertit en molécules organiques solubles qui rejoignent la masse épilimnétique de carbone-14 organique dissous (DOC). Comme la quantité de C^{14} absorbée par les sédiments de l'épilimnion, par l'hypolimnion et par la biomasse périphytonique n'était pas assez importante pour affecter le bilan de masse de C^{14} durant toute l'expérience, le taux d'échange gaseux peut être calculé en mesurant la diminution de l'inventaire de C^{14} épilimnétique ($DIC^{14} + POC^{14} + DOC^{14}$) durant une période de temps définie. Grâce au modèle des limites stagnantes et des valeurs de pCO_2 calculées à partir des données de pH, température et DIC, nous avons obtenu une gamme d'épaisseurs de mince couche stagnante allant de 212 à 316 μm. Afin de vérifier l'épaisseur de la pellicule calculée à partir des inventaires de

[1]Present address: Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6.

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C^{14}, nous avons ajouté à l'épilimnion du lac 224 10 mCi de Ra^{226}. Des mesures de Rn^{222}, fille gaseuzede Ra^{226}, permirent d'évaluer indépendamment l'épaisseur de la pellicule. La valeur moyenne de 200 μm ainsi obtenue s'accorde avec celle que donne la fuite de $C^{14}O_2$. On a aussi construit un modèle décrivant le comportement des masses de POC et DOC. Ce modèle donne des résultats qui s'accordent parfaitement avec le taux de photosynthèse, qui est en moyenne de 65 mg $C \cdot m^{-1} \cdot d^{-1}$, mesuré selon la méthode du C^{14} et de l'incubateur de Fee. Le modèle suggère en outre que seulement environ 10% de la masse POC + DOC est actif dans le processus photosynthétique sur échelle chronologique de 30 jours.

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ALTHOUGH carbon-14 is widely used as a tracer for measuring photosynthetic uptake of carbon by algae (Vollenweider 1974), such measurements have usually been of short duration, conducted in small bottles. As a result, little information is obtained on the long-term fate of added carbon, especially its loss to the atmosphere, hypolimnion, and sediments, or with respect to the long-term role of photosynthetically fixed material in the carbon budget of a whole lake. During the summer of 1976 we added radiocarbon directly to L224, an oligotrophic lake in the Experimental Lakes Area near Kenora, Ontario, to obtain a better idea of some of these interactions and pathways. Radium-226 was also added to give an independent estimate of interaction with the atmosphere by using its gaseous daughter, radon-222.

Description of the Lake

Lake 224 (see fig. 1, Hesslein et al. 1980) has an area of 25.9 ha, a maximum depth of 27 m and a mean depth of 11.6 m. The surface sediments under less than ~10 m of water are primarily sandy with pockets of organic material in depressions. Many areas are also strewn with large stones and boulders. Below 10 m depth, surface, sediments are mostly soft, highly organic ooze. The water is quite clear, bluish green in color with Secchi disc visibility of ~7 m. Chemical characteristics of the water during the experiment are given in Table 1 and 2. Chlorophyll *a* concentration was 0.9–1.1 μg/L. The primary algal species in the epilimnion were flagellated Chrysophyceae (Table 3) and the major herbivores were copepods and Cladocera.

Carbon-14 Pathways in Lake 224

A general scheme for the possible pathways of added C^{14} is shown in Fig. 1. Based on preliminary calculations of epilimnion CO_2 partial pressure from dissolved inorganic carbon (DIC), pH and temperature data, and on some previous gas exchange estimates (Emerson 1975; Torgersen 1977) we believed that evasion of CO_2 to the atmosphere (E) would be a major loss of C^{14} from the epilimnion. Because of the rapid mixing and infinite size of the atmospheric reservoir this loss is irreversible. Another important loss of DIC is due to photosynthetic fixation by algae suspended in the epilimnion (P). This fixed carbon can be (1) released by respiration within the epilimnion (R), (2) lost by settling through the thermocline (S), (3) incorporated in the epilimnion sediments (I), or (4) it could be lost from host cells as a soluble organic molecule by excretion or degradation when algae die or are eaten and become part of the epilimnion dissolved organic carbon (DOC) pool (L). Two other pathways exist but are unimportant in L224. The rate of physical mixing within the thermocline of L224 has been shown to be very small (Quay 1977). The loss of C^{14} by eddy mixing down through the thermocline can therefore be neglected. Because the rate of photosynthesis by periphyton in similar Lake 239 and 240 is small compared to that by algae suspended in the epilimnion (Schindler et al. 1973), uptake of C^{14} by epilimnetic periphyton can be neglected.

TABLE 1. Typical chemical and nutrient composition of Lake 224 epilimnion, Experimental Lakes Area, 1976.

Cl^-	0.6 mg/L	DIC	75 μmol/L
$SO_4^=$	2.6 mg/L	DOC	285 μmol/L
$NO_3^=$	2.0 μg/L	TDP	2 μg/L
HCO_3^- (Calc.)	3.0 mg/L	TDN	225 μg/L
Si	0.2 mg/L	Susp. C	550 μg/L
Na^+	0.75 mg/L	Susp. N	10 μg/L
K^+	0.37 mg/L		
Mg^{++}	0.43 mg/L	Chl *a*	2.7 μg/L
Ca^{++}	1.75 mg/L		

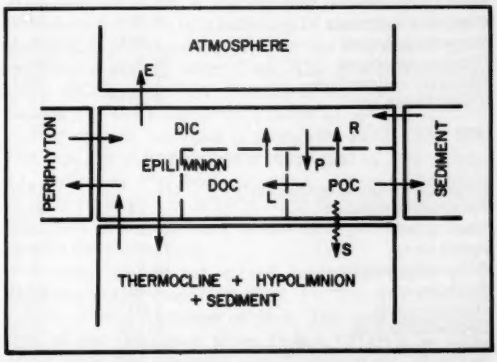

FIG. 1. Possible pathways of carbon-14 in the epilimnion of Lake 224.

TABLE 2. Carbon chemistry of Lake 224, Experimental Lakes Area, 1976.

Date	Day no.	Temperature (°C)	pH	DIC μmol/L	CO_2 (calc.) μmol/L	DOC μmol/L	POC μmol/L
July 26	208	21.7	6.73	76	23.1	249	44
Aug. 9	222	21.1	6.80	70	19.1	228	43
Aug. 23	236	22.0	6.74	70	20.8	306	45
Sept. 6	250	18.0	6.78	86	25.4	300	41

EXPERIMENTAL PROCEDURES

On July 20, 1976, one curie (2.2×10^{12} disintegrations per minute (dpm): (1 Ci = 37 GBq)) of C^{14} in the form of $NaHCO_3$ was transferred to a 20-L drum of lake water (the pH of which had been raised to 11 by the addition of NaOH). This drum was mounted on a raft and towed around the lake while the contents dribbled out through a small copper tube opening to the epilimnion at a depth of 0.5 m. About 45 min was required for the barrel to drain allowing good coverage of the lake area. A tritium injection previously carried out in the epilimnion of nearby Lake 227 (Quay 1977; Quay et al. 1979) showed that horizontal and vertical homogeneity of a tracer added as a point source was achieved within hours. We can be assured that the combination of our geographic dispersal, local mixing by the boat's propeller wash, and natural mixing within the lake thoroughly spread the added C^{14} throughout the epilimnion within a day or two after addition.

Ten millicuries of Ra^{226} in the form of $RaBr_2$ was added to the lake in the same manner as the C^{14}. The Ra^{226} was added as a separate solution of 0.1 N HCl in combination with other isotopes (Hesslein et al. 1980). The Ra^{226} addition was completed within a few hours of the C^{14} addition.

Two limnocorrals, one 10 m in diameter and one 5 m in diameter were deployed in the water column 2 d prior to the isotope additions. Both limnocorrals were 8 m deep and thus penetrated through the thermocline. Both were open at the bottom and both were made of reinforced polyethylene. Separate additions of Ra^{226} were made to the limnocorrals with sufficient amount to reach a similar concentration as in the lake.

TABLE 3. Major components of Lake 224 planktonic community, Experimental Lakes Area.

Species	Phytoplankton 10^4 cells/L	μL/m³ (est.)
July 23, 1976 (Day 206)		
Large Chrysophyceae (300 μm³)	108	323.0
Lyngbya sp.	30.9	92.7
Small greens	62.5	62.5
Pelonema sp.	43.1	43.1
Small Chrysophyceae (50 μm³)	42.0	42.0
Dinobryon bavaricum	4.31	30.2
Botrycoccus braunii	0.095	28.5
Uroglena americana	26.2	26.2
Chroococcus limneticus	25.1	25.1
Gymnodinium spp.	0.057	17.1
Sum		691
August 19, 1976 (Day 232)		
Large Chrysophyceae (300 μm³)	111	334.0
Dinobryon bavaricum	5.74	40.2
Small greens	40.2	40.2
Pelonema sp.	71.2	35.5
Rhizosolenia eriense	16.9	33.8
Syndedra acus	4.46	29.0
Dinobryon spp.	5.03	25.1
Geymnodinium sp.	0.152	22.8
Small Chrysophyceae (50 μm³)	21.6	21.6
Chrysochromulina parva	5.03	15.1
Sum		597

SAMPLE COLLECTION AND PROCESSING

The main emphasis of the C^{14} sampling program was to keep an inventory of the C^{14} in the epilimnion in the forms of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and particulate organic carbon (POC).

At time intervals of 2–6 d water samples (~2 L) for analysis of C^{14} in DIC, DOC, and POC were drawn from the epilimnion through an in-line filter apparatus (Millipore 47 mm Swinnex with Whatman GF/C filter) into an evacuated sample bottle. The sample bottles had 10 mL of concentrated sulfuric acid added prior to evacuation. In the lab, DIC was stripped from the sample by slowly (~30 mL/min) bubbling helium through the acidified sample and directly trapping the stripped CO_2 in a scintillation vial containing a mixture of 2 mL methanol, 2 mL phenethylamine, and 8 mL PCS® (Phase Combining System of Amersham-Searle). A fritted glass end was used to produce small bubbles in the scintillation cocktails. Using standard C^{14} solutions, the $C^{14}O_2$ yield was ~70% after 7–9 h of continuous bubbling with no improvement for longer bubbling times. Faster bubbling rates caused the scintillation cocktail to bubble out of the vial and caused a decrease in the trapping efficiency due probably to larger bubbles. A C^{14} standard was included with each set of samples since the yield varied not only with time but with flow rate. Blank solutions were also tested with samples. A manifold was set up to allow running of six samples simultaneously.

Before digestion of DOC, 7 g potassium persulphate

TABLE 4. Inventory of C^{14} in Lake 224 epilimnion, 1976. (*Unfiltered sample was digested. This probably underestimates $DOC^{14} + POC^{14}$ and overestimates DOC^{14}; **Estimates using primary production model to determine $DOC^{14} + POC^{14}$.)

Δt Days	$(C^{14}/C)_{DIC}$	(C^{14}/C_{DOC})	$(C^{14}/C)_{POC}$	$(C^{14}/C)_{DOC+POC}$	DIC^{14}	DOC^{14}	POC^{14}	$DOC^{14} + POC^{14}$	ΣC^{14}
	← dpm/μmol →				← dpm/L →				
1	23.3	0	0.65	0.097	1699	0	28.2	28.2	1727
1	19.0	0.035	0.40	0.087	1388	8.6	16.7	25.3	1413
6	15.0	0.390	0.72	0.437	1092	96.4	30.9	127.3	1219
6	15.3	0.282	1.21	0.421	1119	70.5	52.0	122.5	1242
8	13.2	0.129	1.53	0.337	962	32.0	66.1	98.1	1060
9	13.9	0.410	NS	0.351	1017	102*		102*	1119
13	10.7	0.286	2.16	0.563	780	71.2	91.6	163.8	944
16	7.3	0.378	2.0	0.617	530	94	85.5	179.5	710
16	7.9	0.341	2.11	0.604	580	85	90.7	175.7	756
22	5.36	0.358	2.18	0.629	391	89.2	93.9	183.1	574
24	7.26	0.369	2.15	0.632	530	92	92.4	184.4	714
28	5.99	0.382	2.11	0.636	437	95.2	90.8	186	623
42	2.38	NS	NS	NS	174	NS	NS	168**	342**

was added and any "left-over" DIC^{14} was removed by evacuation of the sample bottle for about five minutes, causing dissolved gases to boil off with the potassium persulphate crystals acting as boiling chips. Tests demonstrated that the amount of DIC^{14} left in the sample bottle after bubbling evacuation averaged 0.25%. This could only have been a significant contamination in the first few days of the experiment when DIC^{14}/DOC^{14} was large. After evacuation the samples were held at 90°C for 4–6 h in a water bath thus oxidizing the DOC to DIC (Stainton et al. 1977). DIC was then trapped in the above described manner.

The filters containing POC trapped from the above samples were placed in a combustion train consisting of a quartz tube packed alternately with platinum, asbestos, and cupric oxide held at a temperature of 750°C in a stream of pure oxygen (~15 mL/min). POC^{14} was oxidized to CO_2, then trapped in the scintillation vials as above. One analysis required approximately 15 min. C^{14}-tagged algae standards were used to calibrate the system. Overall efficiency of the system was >98%. This combustion system was used rather than direct counting of the uncombusted filters because of interference by other isotopes (Hesslein et al. 1980).

All C^{14} samples trapped in the scintillation cocktail were counted in a cooled liquid scintillation counter (Nuclear Chicago Unilux II). Calibration of sample counts was done by the external standard channels-ratio method. Instrumental error was no more than the ±2%. Total error was no more than ±10%.

The concentrations of DIC and DOC in the lake water were measured in separate samples on the same day as taken. POC samples were dried, frozen, and combusted later. Methods are described by Stainton et al. (1977).

RADON–RADIUM

Radon-222 and radium-226 samples were taken in evacuated bottles (~2 L) to which 10 mL of concentrated hydrochloric acid had been added. The radon gas was then trapped by passing a helium flow through the sample and subsequently a cooled activated charcoal column. The radon was then baked off the column at 470°C and flushed into a phosphor coated counting cell. This cell was then counted on a photomultiplier tube. Radium is done in the same manner after flushing the radon from the sample and allowing a new crop of radium supported radon to be produced. These methods are given in detail by Broecker (1965).

Results

The C^{14} content of DIC in the lake decreased rapidly after addition (Table 4, Fig. 2). The rate of the decrease was about 5.3%/d, or a half-time of about 13 d. DOC^{14} and POC^{14} increased rapidly over the first several days, reaching a plateau after 2 wk. The concentrations of DIC, DOC, and POC remained relatively constant throughout the experiment (Table 2) so that calculation of specific activities is straightforward. DOC and POC specific activities do not reach that of DIC (Table 4), indicating that both receive a substantial portion of their carbon from sources other than the epilimnetic DIC pool or over a much longer time period than covered by the experiment.

Radium-226 in the mixed layer decreased at a logarithmic rate over 60 d (Fig. 3). The loss rate was 1.05%/d or a half-time of 66 d. The zero time intercept of the regression gives 19.9×10^9 dpm as compared with the expected added amount of 22×10^9 dpm. The loss rate of radium-226 is low compared to the other metal isotopes added at the same time (Hess-

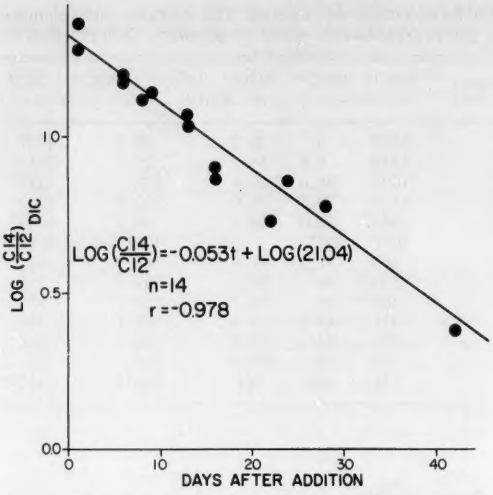

FIG. 2. The carbon-14 content of dissolved inorganic carbon in the epilimnion of Lake 224. The line based on a linear regression of the logarithmic transform of activity against time and parameters for that line are shown.

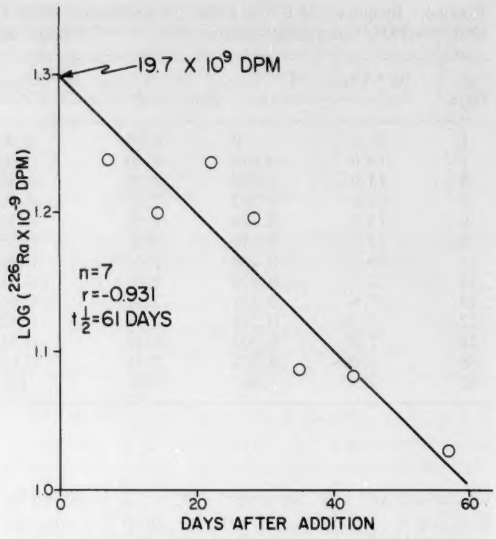

FIG. 3. The radium-226 content of the epilimnion of Lake 224. The line based on a linear regression of the logarithmic transform of amount against time and parameters for that line are shown.

lein et al. 1980). Radon-222 concentrations were remarkably constant with time (Table 5). After 30 d the radon concentration consistently exceeded the radium concentration. This could only have occurred if the radium which had been lost from the water was still contributing radon to the water. This will be discussed below.

GAS EXCHANGE RATE

The gas exchange rate of CO_2 can be calculated from the mass balance of the C^{14} in the lake. As outflow from the lake was zero due to very dry weather the only loss of C^{14} from the whole lake was due to gas evasion. Of losses not included in Fig. 4, downward diffusion is unimportant because the thermocline acts as an effective barrier to rapid diffusion (Quay 1977; Quay et al. 1979) and entrainment of metalimnion waters with time served to return any diffusion C^{14} to the mixed layer. Settling out of POC^{14} to deep water of epilimnion sediments is unknown but can be corrected for by assuming a settling rate of ~0.25 cm/d (Barnes, Canadian Centre for Inland Waters, Burlington, unpublished data; Hesslein et al. 1980). This correction is unimportant initially, because the POC contains no C^{14}.

The gas evasion of CO_2 can be calculated using the ΣC^{14} loss rate, the specific activity of C^{14} in DIC and the area of lake (A).

$$(1) \qquad \frac{\partial \Sigma C^{14}/\partial t}{(DIC^{14}/DIC)A}$$

TABLE 5. Results of Lake 224 whole-lake Ra^{226} spike and calculated gas exchange parameters, 1976.

Δt Days	Mean depth (m)	$[Ra^{226}]$ dpm/L	$[Rn^{222}]$ dpm/L	ΣRa^{226} 10^9 dpm	ΣRn^{222} 10^9 dpm	Rn Obs. / Rn equil.[a]	D/Z (m/d)	Z (μm)
7	5.0	13.4	7.8	17.4	10.1	0.51	0.86	138
14	5.6	11.3	14.8	16.4	20.9	1.05	—	—
22	5.8	11.8	8.7	17.7	13.1	0.66	0.54	219
28	5.9	10.7	9.8	16.4	15.0	0.75	0.35	338
35	5.9	8.0	8.6	12.2	13.2	0.66	0.55	215
43	6.3	7.4	7.8	12.1	12.7	0.64	0.64	185
57	7.0	5.9	6.9	10.7	12.5	0.63	0.74	160
Mean							0.61	194

[a]Rn equil. is a constant 19.94 calculated from the regression of Ra^{226} versus time (Fig. 3).

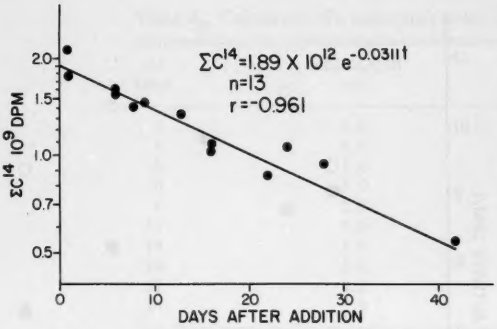

FIG. 4. Total carbon-14 in epilimnion of Lake 224. The line based on a linear regression of the log transform of the amount against time and parameters for that line are shown.

where $\Sigma C^{14} = DIC^{14} + DOC^{14} + POC^{14}$ in dpm. Specific activity is in dpm/mol and A in m^2.

As shown in Fig. 4, the ΣC^{14} in the lake water can be well represented by the equation

$$\Sigma C^{14} = 1.89 \times 10^{12} e^{-0.0311t} \quad (2)$$

where t is in days and ΣC^{14} in dpm. DIC^{14}/DIC can be represented as in Fig. 2.

$$DIC^{14}/DIC = 21.04 e^{-0.053t} \quad (3)$$

The area of the lake is 25.92×10^4 m^2. Substituting into equation (1) yields:

$$E = \frac{0.0311(1.89 \times 10^{12})\, e^{-0.0311t}}{21.04\, e^{-0.053t}\, 25.92 \times 10^4} \frac{\text{mole}}{m^2\ \text{day}} \quad (4)$$

Gas evasion rates calculated from this equation are shown in Table 6. Correction for settling particulate is given by

$$\frac{POC^{14}\, S}{DIC^{14}/DIC} \frac{\text{mole}}{m^2\ \text{day}} \quad (5)$$

where S is the settling rate in metres per day. To calculate the correction POC^{14} values were taken from measured data (Table 4), S was taken as 0.25 m/d and DIC^{14}/DIC generated by equation 1. This correction reduces the apparent increase in gas evasion values by about 50% (Table 6). If $POC^{14} + DOC^{14}$ values (Table 4) are used to represent the settling particulate instead of POC^{14} alone the entire apparent increase in gas evasion disappears (Table 6). The use of $POC^{14} + DOC^{14}$ may be more correct as we suspect that at least part of the DOC^{14} is an artifact due to filtering (see following section on photosynthesis).

From a knowledge of the dissolved gaseous CO_2 content of the epilimnetic water (as calculated from pH and DIC results) the stagnant film thickness, z, and the mass transfer coefficient, D/z, can be obtained.

$$z = \frac{D\{CO_2\}}{E} \quad (6)$$

$$\frac{D}{z} = \frac{E}{\{CO_2\}} \quad (7)$$

where D is the molecular diffusion coefficient of CO_2 in water of 1.9×10^{-5} cm^2/s. These values are given in Table 6.

The mean value of gas evasion using only the POC^{14} correction is 1.31×10^{-2} and 1.06×10^{-2} $mol \cdot m^{-2} \cdot d^{-1}$ using the $POC^{14} + DOC^{14}$ correction. Using the calculated value of CO_2 gas concentration from Table 2, these yield respectively boundary thicknesses of 260 and 321 μm, and mass transfer coefficients of 0.62 and 0.50 m/d. Some measurements of CO_2 gas concentration have been made in L224 (R. B. Cook, Lamont-Doherty Geo. Obs., personal communication) which do not agree with calculated concentration values. If L224 is assumed to be at equilibrium with the atmosphere it would have a CO_2 gas concentration of 12.5 μmol/L. This would give boundary thicknesses of 155 and 191 μm. The mass transfer coefficients would be 1.04 and 0.84 m/d.

TABLE 6. Calculated gas evasion from C^{14} loss.

Δt Days	Raw CO_2 gas evasion $mol \cdot m^{-2} \cdot d^{-1}$	Corrected gas evasion ($mol \cdot m^{-2} \cdot d^{-1}$)		Mass transfer coefficient (m/d)			
				CO_2 = 21 μmol/L		CO_2 = 12.5 μmol/L	
		POC^{14} only	$POC^{14} + DOC^{14}$	POC^{14}	$POC^{14} + DOC^{14}$	POC^{14}	$POC^{14} + DOC^{14}$
1	1.10×10^{-2}	1.07×10^{-2}	1.07×10^{-2}	0.51	0.51	0.86	0.86
6	1.23×10^{-2}	1.16×10^{-2}	1.03×10^{-2}	0.55	0.49	0.93	0.82
11	1.37×10^{-2}	1.22×10^{-2}	1.06×10^{-2}	0.58	0.50	0.98	0.85
16	1.53×10^{-2}	1.29×10^{-2}	1.04×10^{-2}	0.61	0.50	1.03	0.83
21	1.71×10^{-2}	1.38×10^{-2}	1.06×10^{-2}	0.66	0.50	1.10	0.85
26	1.90×10^{-2}	1.47×10^{-2}	1.04×10^{-2}	0.70	0.50	1.18	0.83
31	2.13×10^{-2}	1.59×10^{-2}	1.05×10^{-2}	0.76	0.50	1.27	0.84
Calculated mean mass transfer coefficient (m/d)				0.62	0.50	1.04	0.84
Calculated mean boundary layer thickness (μm)				259	321	155	191
$D = 1.9 \times 10^{-5}$ cm^2/s							

TABLE 7. Ra^{226} and Rn^{222} in limnocorrals in Lake 224, 1976.

Δt Days	Mean depth (m)	[Ra^{226}] dpm/L	[Rn^{222}] dpm/L	Rn^{222}/Ra^{226}	D/Z (m/d)	Z (μm)
5 m diameter limnocorral						
17	7.0	8.81	8.06	0.91	0.125	950
22	7.2	10.93	7.46	0.68	0.61	194
28	7.5	12.76	9.59	0.75	0.45	263
35	7.0	10.44	6.34	0.61	0.80	148
43	7.5	8.58	4.48	0.52	1.24	95
Mean	7.3	—	—	0.69	0.58	206
10 m diameter limnocorral						
43	7.5	7.5	4.8	0.64	0.73	163

The gas evasion rate of radon-222 is calculated from the amount of radon "missing" from the water relative to its radioactive parent radium-226. The mass transfer coefficient can be expressed as:

$$(8)\quad \frac{D}{z} = \left(\frac{1}{\frac{C}{C_e} - 1}\right)h\lambda \quad \text{(Broecker and Peng 1974)}$$

where C_e is the equilibrium concentration of radon, C is the measured concentration of radon, h is the mean depth, and λ is the decay constant of radon-222, 0.18 d^{-1}. The piston velocity calculated from radon deficiency represents the gas exchange mean over a few days before measurement. For the equation to be meaningful C/C_e must be ≤ 1. As can be seen in the data (Table 5) this is not always the case in the lake. The only explanation for this is that the radium which has been lost to the sediments still contributed its radon to the water. As a precise estimate of the efficiency of this contribution is not possible, only an upper limit can be placed on the effective C_e, that is, to assume that all radon produced does get into the epilimnion. This also gives an upper limit on the gas exchange rate. Support for the assumption that all radium went to the epilimnetic sediments comes from evidence in a similar experiment done in L227 (Emerson and Hesslein 1973), where radium-226 went initially to epilimnetic sediments, but did not appear in the metalimnion or hypolimnion. Radium analyses of L224 sediments have not yet been completed. In the limnocorrals there is no complication due to radium loss and the calculation of gas evasion is straightforward.

The values of mass transfer coefficient and boundary thickness (Table 5) are calculated for the lake based on a total radium content equal to that added, as determined by the intercept of the regression in Fig. 3. The values for the limnocorrals (Table 7, Fig. 5) are calculated directly from the data. Mean values are calculated from the mean equilibrium values not from the mean of the gas evasion coefficients. The mean value for the mass transfer coefficient and boundary thickness for the 5 m diameter limnocorral are 0.58 m/d and 206 μm, respectively. The corresponding values for the 10 m diameter limnocorral are 0.73 m/d and 163 μm.

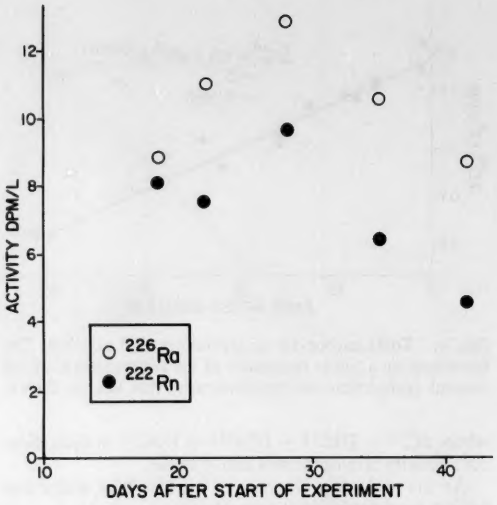

FIG. 5. The concentrations of radium-226 and radon-222 in the 5 m diameter limnocorral in Lake 224.

Discussion

All determinations of the gas exchange parameters for L224 are in good agreement considering the differences in physical structures and the lack of precise understanding of the pathways of radium and radon. The boundary layer thicknesses calculated from the small and large limnocorrals of 206 and 163 μm, respectively, agree quite well with the value of 194 μm determined by the whole-lake radium–radon experiment. The radium–radon determined values agree well with the C^{14} determined value assuming CO_2 on the lake is in equilibrium with the atmosphere and using $POC^{14} + DOC^{14}$ as representative of carbon settling. This value is 193 μm. A whole lake value of about 200 μm can be compared to that determined for L227 by Emerson (1975) of 300 μm. Torgersen (1977) using the He^3–H^3 method estimates the L227 boundary thickness at 600–1000 μm. As wind stress is higher on the larger L224, the higher gas exchange there is not surprising.

Implications of Gas Exchange to the Lake's Carbon Balance

As mentioned (Table 2), the calculated dissolved CO_2 gas content of the epilimnion of L224 (21 μmol/L) is 1.7 times that for equilibrium with the atmosphere (12.5 μmol/L). This would require that CO_2 must be evading the lake at 1.7 times the rate that it is invading.

TABLE 8. Calculation of a lower limit to the rate of photosynthesis (P — in term of C), 1976.

Δt Days	Mean depth (m)	$(C^{14})_{DOC+POC}$ 10^9 dpm/m²	$(C^{14}/C)_{DIC}$ dpm/μmol
1	4.8	0.135	23.3
1	4.8	0.121	19.0
6	5.0	0.636	15.0
6	5.0	0.613	15.3
8	5.2	0.510	13.2
13	5.6	0.918	10.7
16	5.6	1.01	7.30
16	5.6	0.986	7.91
22	5.8	1.061	5.36
24	5.9	1.07	7.26

Period (days)	$(C^{14})_{DOC+POC}$ 10^9 dpm/m²	(Mean $C^{14}/C)_{DIC}$ dpm/μmol	Δt Days	P min 10^{-3} mol·m^{-2}·d^{-1}	P min mg·m^{-2}·d^{-1}
1–6	0.497	18	5	5.5	66
6–16	0.365	11.5	10	3.1	38
16–24	0.072	7.5	8	1.3	16

This leads to a net loss to the atmosphere of 5.4×10^{-3} mol·m^{-2}·d^{-1}. Over the 42-d period of the experiment this would have been 0.23 mol/m² or 0.038 mol/m³ of DIC lost. As shown in Table 2 the concentration of DIC did not change significantly over this time interval. The amount of DIC lost to the atmosphere must have therefore been replenished from some other pool in the lake. Three possible sources exist: net respiration in the epilimnetic water, entrainment of DIC by epilimnetic deepening, and respiration in the epilimnetic sediment. No consistent change in the concentration of epilimnetic POC occurred during the experiment. If any, the trend is for DOC concentration to increase. To produce the CO_2 necessary to replace that lost by the gas evasion, at least 10% of the combined pool of POC + DOC would have to have been oxidized (Table 8). This argues against net respiration in the epilimnion. DIC concentrations below the epilimnion to 10 m depth were no higher than the values for the epilimnion given in Table 2, so entrainment could not have supplied sufficient additional DIC. Some flux of DIC from epilimnetic sediments does undoubtedly occur. To compensate for the losses to the atmosphere of 5.4×10^{-3} mol·m^{-2}·d^{-1} at the surface of L224, the flux from the epilimnetic sediments would have to be 18×10^{-3} mol·m^{-2}·d^{-1} (the area of sediment is ~30% of the surface area of the lake). Sediment fluxes of DIC have been measured in L227 at 26.5×10^{-3} mol·m^{-2}·d^{-1} (Hesslein 1976). Lake 227 is far more productive than L224 and this flux is probably greater than could be expected in L224.

The validity of this calculation is questionable because of the uncertainty in the accuracy of the calculated concentration of CO_2 gas. If the dissolved CO_2 gas is at equilibrium with the atmospheric CO_2, as is suggested by the agreement of gas exchange values calculated using this assumption with those determined by the radium–radon method, no net carbon flux will occur. It is clear than an accurate direct measurement of dissolved CO_2 gas is required to calculate the net carbon flux.

THE RATE OF PHOTOSYNTHESIS

The rate of photosynthesis over the period of the experiment can be determined from the appearance of C^{14} as organic carbon POC and DOC pools. A lower limit of the rate of photosynthesis can be calculated by assuming that all the C^{14} fixed remained in the epilimnetic DOC and POC pools during the period of the calculation (i.e. that loss by respiration and settling were small). The rate of photosynthesis can then be represented as:

$$(9) \quad P = \frac{\partial h(POC^{14} + DOC^{14})}{\partial t} \Big/ DIC^{14}/DIC \times 12 \times 10^3 \frac{mg}{mol}$$

where P is the rate of photosynthesis in mg·m^{-2}·d^{-1},
$POC^{14} + DOC^{14}$ is the concentration of C^{14} in POC + DOC in dpm/m²,
h is the mean depth of the mixed layer,
DIC^{14}/DIC is the specific activity of DIC in dpm/mol,
t is time.

Table 8 shows the results of these calculations for three time periods using linear averages over those periods. As can be seen the rate of photosynthesis drops off with time. This is probably an artifact due to the assumption that respiration and settling are negligible. The photosynthesis value for the 6 d, 66 mg·m^{-2}·d^{-1} is not affected as greatly by this assumption, because there was little C^{14} in the POC or DOC pools to be respired or settled at that time. This value can be compared with an average value of 56 mg·m^{-2}·d^{-1} obtained for

this 6-d time interval by Fee (unpublished data) using the C^{14} technique with bottles in an incubator (Fee 1973).

A model can be constructed which incorporates respiration and settling with the introduction of some assumptions and one new parameter. The appearance of C^{14} in the (DOC + POC) pool can be:

$$(10)\quad \partial\left\{h\frac{POC^{14}+DOC^{14}}{POC+DOC}\times(POC+DOC)\right\}\Big/\partial t = P(DIC^{14}/DIC) - \frac{(R+S)(POC^{14}+DOC^{14})}{f(POC+DOC)}$$

where R is respiration, S is settling, and f is the fraction of the POC + DOC which is actually labeled with C^{14} by the photosynthetic processes in the lake. To solve the above equation it is assumed that the rate of respiration plus settling is equal to the rate of photosynthesis, i.e. there is no net change in organic carbon in the epilimnion, and that the respired or settled material is characterized by the specific activity of epilimnetic POC + DOC. The first assumption is supported by the POC and DOC data (Table 2) and without further information is the only reasonable one. The fraction f allows for the possibility that a portion of the DOC and POC pools may not participate in photosynthesis respiration and settling. Such components as dead material or stable humic and fulvic acid compounds might be examples of organic material excluded by f. The specific activity of "active" DOC + POC is thus given by

$$(11)\quad \frac{(POC^{14}+DOC^{14})}{f(POC+DOC)}$$

As shown in Fig. 2 the C^{14}/C^{12} ratio in DIC is well fitted by the equation $DIC^{14}/DIC = 21.04e^{-0.053t}$. This yields:

$$(12)\quad \frac{\partial[(POC^{14}+DOC^{14})/(POC+DOC)]}{\partial t} = \frac{P\{21.04\exp(-0.053t)\} - \dfrac{(POC^{14}+DOC^{14})}{f(POC+DOC)}}{h(POC+DOC)}$$

As h is function of time this model can be solved most easily as a numerical model on a computer. The numerical solution allows the possibility of varying P with time as well as h. The vaue of f is set so that the maximum values of $(POC^{14}+DOC^{14})/(POC+DOC)$ generated by the model is equal to the maximum value in the data.

Two approaches were taken to numerically produce the $(POC^{14}+DOC^{14})/(POC+DOC)$ curve from the model. Figure 6 shows a model generated curve produced by using daily values of P as measured by Fee (unpublished data) with the incubator technique. As can be seen the fit to the data is very good. The second approach was to hold P constant throughout the time period. The results using P = 40, 60, and 80 mg·m^{-2}·d^{-1} are shown in Fig. 7. The average value of photosynthesis as determined by Fee was 65 mg·m^{-2}·d^{-1}. The 60 mg·m^{-2}·d^{-1} curve looks very much like the one generated using daily values in Fig. 6. The shapes of the curves for 40 and 80 mg·m^{-2}·d^{-1} are rather different than the data.

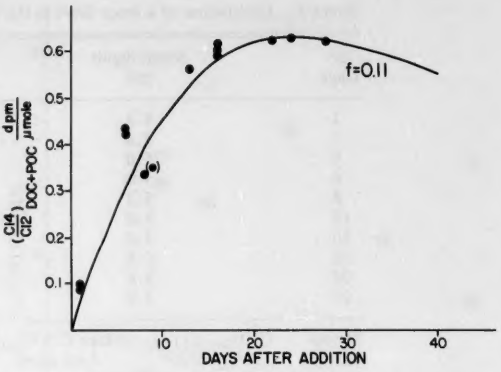

FIG. 6. Carbon-14/carbon-12 ratio of the organic carbon pool (particulate plus dissolved) of the epilimnion of Lake 224. The curve is that generated by the primary production model using the production data of Fee and an active fraction of 0.11 (see text).

The meaning of the fraction f is difficult to determine because it characterizes the combined pool of POC + DOC. At the end of 30 d the specific activity (C^{14}/C^{12}) of POC reaches 0.35 of the specific activity of DIC. The DOC specific activity is 0.06 of the DIC specific activity. Because the shape of the curve of POC^{14} + DOC^{14} and the model suggest that the system is nearly at equilibrium by this time, these numbers can be inter-

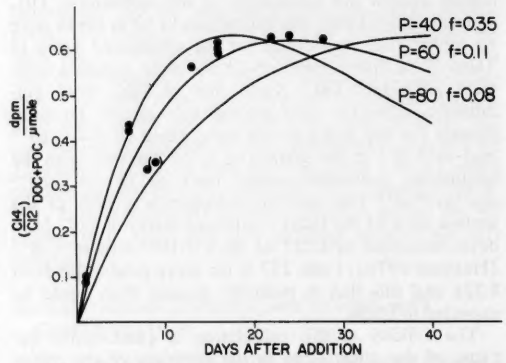

FIG. 7. Three curves generated by the primary production model and parameters for the model (see text) are shown with the carbon-14/carbon-12 ratios of the organic carbon pool (particulate plus dissolved) of the epilimnion of Lake 224.

preted to mean that 35% of the POC and 6% of the DOC are involved in the short term (<100 d) photosynthetic carbon pool of the lake. Conversely this leads to the conclusion that 0.65 of POC and 0.94 of the DOC are either allocthonous or long in equilibrating with lake carbon. The separate determination of POC^{14} and DOC^{14} is far from certain, however, as we believe part of distinction between POC^{14} and DOC^{14} may be an artifact of filtration produced by cell breakage or leakage of dissolved organic material from cells. We have some evidence that at least part of this separation is artifact (P. Bower, Lamont-Doherty, Geo. Obs., personal communication). This would explain the observation (Table 4) that replicates of (DOC^{14} + POC^{14}) agree more closely than DOC^{14} or POC^{14} replicates alone. Using the data as (POC^{14} + DOC^{14}) in the model is advantageous because it allows direct comparison with Fee's data. Fee's samples are not filtered, but counted after DIC^{14} has been sparged by acidifying and bubbling with air (Schindler et al. 1972).

The two major results of this whole lake radiocarbon experiment are that if dissolved CO_2 gas calculations are correct, the lake is losing carbon at a rate of 5.4×10^{-3} mol·m^{-2}·d^{-1} (1.4×10^{3} mol·d^{-1}) and that photosynthetic production, while well estimated by the C^{14} incubator technique of Fee, seems to be responsible for only a small part of the organic carbon in the water. Because the chemical conditions controlling the concentration of gaseous CO_2 in the epilimnion (pH, DIC, temperature) do not vary greatly over most of the ice free season (D. W. Schindler, unpublished data) the yearly loss of carbon by net gas evasion can be estimated at ~1 mol·m^{-1}·yr^{-1}. This net loss of carbon to the atmosphere demands that carbon fixed in photosynthesis be derived from a source other than the atmosphere. Recent carbon isotopic measurements (Rau 1978) suggest that this is the case in other lakes. The two possible sources for this carbon are the sediments and inflow (rainfall, runoff, etc.). If the sediments are net accumulators of carbon, as suggested by Kipphut (1978) in a study of sedimentation in other oligotrophic lakes in the Experimental Lakes Area, the only source is inflow. The carbon added by runoff, rainfall, and dryfall must be organic, because DIC levels in these components are very low due to the absence of carbonate minerals in the area. Such an addition of terrestrial organic material could explain the large fraction of POC and DOC which apparently is not a result of photosynthesis in the lake.

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Gas Exchange, Photosynthetic Uptake, and Carbon Budget for a Radiocarbon Addition to a Small Enclosure in a Stratified Lake[1]

PETER BOWER AND DANIEL MCCORKLE

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York, NY 10964, USA

BOWER, P., AND D. MCCORKLE. 1980. Gas exchange, photosynthetic uptake, and carbon budget for a radiocarbon addition to a small enclosure in a stratified lake. Can. J. Fish. Aquat. Sci. 37: 464–471.

9250 kBq (250 μCi) of ^{14}C as $NaHCO_3$ were added to the mixed-layer waters inside a long, cylindrical plastic enclosure anchored in an oligotrophic lake of the Canadian Shield. Loss of ^{14}C from the epilimnion was predominantly in the form of irreversible gas-exchange across the liquid–air interface. This loss was measured by ^{14}C inventory of the epilimnion and thermocline waters. Using the Lewis and Whitman boundary layer model, values for the mass transfer coefficient of 126, 58, and 100 cm/d were determined for three distinct phases in the deepening of the epilimnion during autumn cooling. The relationship between these mass transfer coefficients and the average wind speeds over the same three time periods were consistent with the results of previous wind-tunnel, gas-exchange experiments.

Two significant features of the carbon budget during the course of the experiment were the large net outflux of CO_2 from the corral (with P_{CO_2} in the epilimnion 3–7 times atmospheric levels) and the doubling of the total dissolved inorganic carbon (DIC) content of the epilimnion. The major source of carbon for these two processes was the entrainment of dissolved inorganic carbon as the epilimnion deepened during the cool days of late summer. Particulate organic carbon was also entrained and its oxidation contributed to the net DIC increase and CO_2 loss. Simultaneous determinations of daily integral primary productivity by an incubator technique and by direct measurement of ^{14}C uptake inside the enclosure were consistent. Dark respiration was 45–53% of daily integral primary productivity, but total respiration was nearly two times that for dark plus light respiration. Net primary productivity was thus substantially negative.

Key words: Gas exchange, photosynthetic uptake, carbon budget

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On a ajouté 9250 kBq (250 μCi) de ^{14}C sous forme de $NaHCO_3$ aux eaux de la couche mélangée à l'intérieur d'une longue enceinte cylindrique en plastique ancrée dans une lac oligotrophe du Bouclier canadien. La perte de ^{14}C de l'épilimnion se produit surtout sous forme d'un échange gaseux irréversible à travers l'interface liquide–air. Nous avons mesuré cette perte par dosage du ^{14}C dans les eaux de l'épilimnion et de la thermocline. Le modèle des couches limites de Lewis et Woodman a servi à déterminer les valeurs du coefficient de transfert de masse de 126, 58 et 100 cm/jour pour trois phases distinctes de l'épaississement de l'épilimnion pendant le refroidissement automnal. La relation entre ces coefficients de transfert de masse et les vitesses moyennes du vent durant ces trois périodes s'accorde avec les résultats d'expériences antérieures d'échange de gas dans un tunnel aérodynamique.

Au cours de l'expérience, le budget du carbone montra deux importantes caractéristiques: abondant efflux net de CO_2 de l'enceinte (avec P_{CO_2} dans l'épilimnion de 3 à 7 fois les niveaux atmosphériques) et doublement du contenu en carbone inorganique dissous (DIC) de l'épilimnion. La principale source de carbone pour ces deux processus est l'entraînement de carbone inorganique dissous à mesure que l'épilimnion s'épaissit durant les jours frais de fin d'été. Le carbone organique particulaire est également entraîné et son oxydation contribue à l'augmentation nette du DIC et à la perte de CO_2. Des déterminations simultanées de productivité primaire intégrale quotidienne par incubation et par mesures directes d'assimilation de ^{14}C dans l'enceinte sont en accord. La respiration à la noirceur est de 45 à 53% de la productivité primaire intégrale quotidienne, mais la respiration totale est presque deux fois celle de la respiration à la noirceur et à la clarté. La productivité primaire nette est donc fortement négative.

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SINCE its introduction in the 1950s the ^{14}C method for measurement of primary productivity (Steeman-Nielsen 1952) has undergone numerous modifications and advances. Both in situ and incubator methods have been developed (Schindler 1966; Strickland and Parsons 1968; Vollenweider 1969; Gargas 1975; Gargas et al. 1976; Fee 1973; Steeman-Nielsen 1977). When proper attention is given to methodological pitfalls, particularly to the measurement of excreted dissolved organic carbon (Thomas 1971; Schindler et al. 1972a) current techniques can provide reliable and precise information. These techniques, however, must assume that lake water samples enclosed in small bottles accurately reproduce the natural conditions of the lake environment. Evidence has been presented (Venrick et al. 1977) that this assumption may not always be justified, particularly for incubations longer than a few hours. One purpose of the ^{14}C addition to a large mass of enclosed epilimnetic water, was to allow the simultaneous measurement and comparison of primary production estimates from an incubator (bottle) technique and from direct measurements of ^{14}C uptake in the enclosed, natural waters.

The ability to estimate the flux of chemical species across a liquid–air interface is critical for the budgets of many elements and thus for a proper understanding of their dynamics in natural water systems. Only with accurate measurements of net gas-exchange was it possible to demonstrate that the invasion of CO_2 from the atmosphere could entirely support the carbon requirements of a highly euthrophic lake (Schindler et al. 1972b). Carbon budgets are also important to lake management, particularly in the assessment of oxygen consumption or depletion. Understanding the nature of carbon flux through food chains has been important to the classical study of trophic structure and function of lake ecosystems (Goldman 1968). An overall understanding of carbon cycling must take into account the relationship and interaction of lake biology and geochemistry and is better developed in the framework of well-defined and accurate carbon budgets. This is well illustrated in the limitations placed on carbon budgets for Lake Ontario by uncertainties in the estimates of gas-exchange (Charlton 1977). Consequently, the other purpose of the radiocarbon addition to the enclosed lake water was to more accurately define CO_2 gas flux with particular emphasis on its role in determining the overall carbon budget of the system.

Materials and Methods

During the summer of 1977 a large cylindrical, heavy-walled, plastic enclosure (limnocorral) was anchored to an immobile flotation collar in the deepest part of the South Basin of Lake 302 (Brunskill and Schindler 1971), a dimictic, oligotrophic body of water in the Canadian Shield and part of the Canadian Government's Experimental Lakes Area (ELA) near Kenora, Ontario (Schindler and Fee 1974). The limnocorral, with a diameter of 3.5 m and a length of 8 m, extended below the thermocline of L302 and due to the flotation collar, which extended nearly 15 cm above the lake surface, provided a large isolated volume of epilimnetic lake water.

9250 kBq (250 μCi) of $NaH^{14}CO_3$ were added to the epilimnion of the limnocorral on the evening of August 7, 1977 (day 219). Sampling commenced early in the morning on day 220. 155.4 kBq (4.2 μCi) of ^3H_2O were also added to measure the volume of the corral, to monitor mixing, to test for leakage, and to measure any changes in the depth (Z_e) of the limnocorral epilimnion. Just before the addition of the $NaH^{14}CO_3$, four sediment traps were placed at a depth of 7 m within the limnocorral. Strips of plastic made from the same material as the limnocorral were hung along the sides and across the corral for study of ^{14}C uptake by periphyton. Both the plastic strips and the limnocorral had fresh, clean surfaces when emplaced 2 d before the $NaH^{14}CO_3$ addition.

Measurements of total dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), particulate organic carbon (POC), and pH, were made at the ELA analytical lab within a few hours of collection (Stainton et al. 1977). Analytical techniques for measurement of ^{14}C from DIC, DOC, and POC fractions were the same as those described for the whole-lake radiocarbon experiment (Hesslein et al. 1979). Wind speed measurements were made at the ELA Meteorological station (about 2 km from L302) with a Type 45B MSC Anemometer mounted on a 12-m tower.

The rate of net gas transport or flux (F), across a liquid–air interface was calculated according to the stagnant film or boundary layer model (Lewis and Whitman 1924; Dankwerts 1970; Broecker and Peng 1974; Emerson 1975a) as

$$(1)\quad F_{net} = F_{in} - F_{out} = \frac{D([CO_2]_I - [CO_2]_L)}{z}$$

where D is the coefficient of molecular diffusion for the particular gas, $[CO_2]_I$ is the equilibrium concentration of the dissolved gas at the liquid–air interface, $[CO_2]_L$ is the concentration of the dissolved gas below the boundary layer, and z is the boundary layer thickness. The rate of CO_2 gas-exchange was determined by measuring the decrease in ^{14}C within the epilimnion of the limnocorral as a function of time.

The mass transfer coefficient or piston velocity, D/z, can be calculated from the following relation

$$(2)\quad F_{out} = Z_e \cdot \frac{d\Sigma^{14}C_{EPILIMNION}}{dt} = \frac{D \cdot [CO_2]_L(^{14}C/C)_{DIC}}{z}$$

where $\Sigma^{14}C_{EPILIMNION}$ is the epilimnetic inventory of $DO^{14}C + PO^{14}C + DI^{14}C$, and $(^{14}C/C)_{DIC}$ is the specific activity of ^{14}C in DIC. In integral form this becomes:

$$(3)\quad \int_{t_1}^{t_2} d\Sigma^{14}C_{EPILIMNION} = \frac{D}{z}\int_{t_1}^{t_2} \frac{[CO_2]_L \cdot (^{14}C/C)_{DIC}dt}{Z_e}$$

where Z_e, $[CO_2]_L$, and $(^{14}C/C)_{DIC}$ are all functions of t. CO_2 dissolved in the liquid phase, or $[CO_2]_L$, can be calculated from pH, temperature, and DIC data. Since the pH remains close to 6.4

$$(4)\quad [CO_2]_L + [HCO_3^-] = \Sigma DIC.$$

Given the equilibrium relation,

$$(5)\quad \frac{[HCO_3^-][H^+]}{[CO_2]_L} = K_1$$

and the temperature dependence of K_1 (Stumm and Morgan 1970), $[CO_3]_L$ can be calculated from the equation

$$(6)\quad [CO_3]_L = \frac{\Sigma DIC}{\frac{K_1}{[H^+]} + 1}$$

The net mass of carbon lost from the limnocorral by gas-exchange may be determined by solving the following integral:

$$(7)\quad F = \frac{D}{z}\int_{t_1}^{t_2} [CO_2]_I - [CO_2]_L dt$$

where $[CO_2]_I$ is the concentration of dissolved CO_2 in the liquid phase in equilibrium with the ambient atmospheric CO_2 partial pressure. $[CO_2]_I = 12.5\ \mu$mol/L for $P_{CO_2} = 3.2 \times 10^{-4}$ atm (Stumm and Morgan 1970).

Measurements of daily integral primary productivity (P) were made at ELA by an incubator technique (Fee 1973). Simultaneous measurements of the same parameter were made by direct sampling of the limnocorral epilimnion and were calculated from the following equation

$$(8)\quad P = \frac{Z_e(\Delta\Sigma^{14}C_{ORGANIC})}{\Delta t(^{14}C/C)^*_{DIC}}$$

where $(^{14}C/C)^*_{DIC}$ is the average specific activity of DIC during the day and $\Delta\Sigma^{14}C_{ORGANIC}$ is the change in ($PO^{14}C + DO^{14}C$) over the period of time, Δt. Terms like net primary productivity (PPnet), net photosynthesis (Pn), gross photosynthesis (Pg), and their variations are often misused or improperly defined. Along with mineralization (m) and daylight (Rd) and nightime (Rn) respiration, this paper has followed the terminology of Golterman (1975), such that PPnet = Pg − Rd − Rn − m = Pn − Rn − m.

Results

Vertical profiles of $DI^{14}C$ and ^3H_2O showed that complete and uniform mixing of both the $NaH^{14}CO_3$ and ^3H_2O additions took place overnight down to a depth of 5.2 m, the depth of the top of the thermocline at the beginning of the experiment. Vertical profiles of ^3H_2O were also used to monitor changes in Z_e due to deepening of the epilimnion during the early stages of fall overturn (Fig. 1). From the known activity of ^3H_2O initially added, the volume of the corral was calculated to be 49.6 m^3. No leakage of the limnocorral was detected based on calculations of mass balance of ^3H_2O vs. time.

Uptake of ^{14}C by periphyton was negligible, and the $\Sigma^{14}C$ content of the POC from the four sediment traps (measured on day 237 and adjusted for surface area) was less than 0.4% of the original spike (specific activity = 1.3 dpm/μmol). There were, therefore, no significant losses of ^{14}C from the limnocorral other than gas exchange.

Gas Exchange

The mass transfer coefficient or piston velocity, D/z, for gas exchange was calculated from equation 3 using

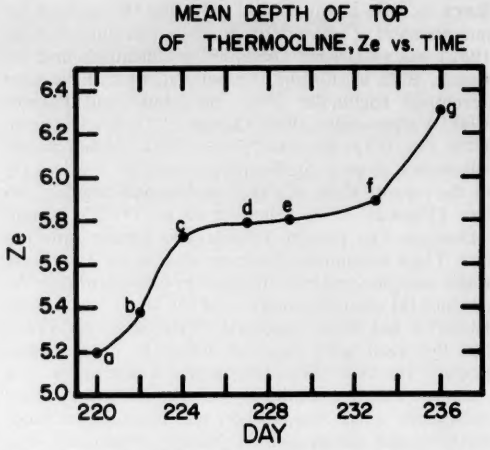

FIG. 1. Depth of H^3 penetration or mean depth of top of thermocline (Z_e) vs. time.

the data presented in Tables 1 and 2 to define the time dependent functions $[CO_2]_L$, and $(^{14}C/C)_{DIC}$. Thus, $(^{14}C/C)_{DIC} = 121.9\ e^{-0.1247(t-220)}$ with $r = -0.98$ (Fig. 2) and $[CO_2]_L = 36.99 + 3.19\ (t\text{-}220)$ with $r = 0.98$. Three distinct phases in the change of Z_e vs. t (Fig. 1) are defined by line segments: a–c, days 220–224, $Z_e = 5.20 + 0.133(t\text{-}220)$; c–f, days 224–223, $Z_e = 5.73 + 0.018(t\text{-}224)$; and f–g, days 233–236, $Z_e = 5.89 + 0.140(t\text{-}233)$. The three separate integrals associated with these time periods were solved sequentially by parts giving $D/z = 126$, 58, and 100 cm·d^{-1}, respectively. Since $D = 1.64 \times 10^{-5}$ cm^2·s^{-1}

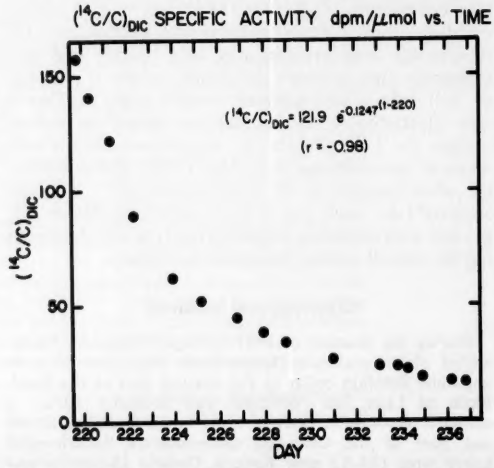

FIG. 2. Specific activity of ^{14}C in dissolved inorganic carbon vs. time.

TABLE 1. Laboratory measurements for dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), particulate organic carbon (POC), and pH, of field measurements of temperature, and of calculations for dissolved aqueous CO_2 ($[CO_2]_L$) using equilibrium constant (K_1) data from Stumm and Morgan (1970). (*Interpolated.)

Day	Temp. (°C)	POC (μmol/L)	DOC (μmol/L)	DIC (μmol/L)	$[CO_2]_L$ (μmol/L)	pH	$-\log K_1$
220 A.M.	20.5	78	418	69	34.7	6.39	6.377
220 P.M.	20.5	62	425	70	35.2	6.60	6.377
221	20.3*	73	500	73	36.8	6.79	6.378
222	20.1*	71	510	91	45.9	6.52	6.379
224	19.8*	73	410	106	53.6	6.40	6.382
227	19.2	87	397	126	64.0	6.20	6.385
229	19.0*	91	700	136	69.3	6.30	6.387
231	18.6*	—	—	—	—	—	—
234	18.0	68	470	152	78.1	6.40	6.394
235	17.9*	83	425	161	82.8	6.49	6.395
236	17.8*	82	—	159	81.8	6.20	6.396
237	17.7	77	455	142	73.2	6.25	6.397

for CO_2 at 20°C (Broecker and Peng 1974), the respective average boundary layer thicknesses were 112, 245, and 142 μm. Chemical enhancement of CO_2 gas-exchange (Emerson 1975b) by a factor α may be estimated from the experiments of Hoover and Berkshire (1969). In the pH range 6.37–6.74 and at a film thickness of 181 μm, they observed α to be 1.12. Using the new mass transfer coefficient, $\alpha D/z$, z valves were recalculated to be 125 μm (days 220–224), 275 μm (days 224–233), and 160 μm (days 233–236).

Average wind speeds and the corresponding mass transfer coefficient for each of the three phases of epilimnetic deepening were:

Time period (Day of year)	220–224	225–233	233–236
Average wind speed (m/s)	3.12	2.19	2.46
Mass transfer coefficient D/z (cm/h)	5.25	2.42	4.17

CARBON BUDGET

The net flux of CO_2 (F_{net}) was determined using equation 7. Thus, $F_{net} = -35$ mmol $C \cdot m^{-2} \cdot d^{-1}$ (days 220–224), -26 mmol $C \cdot m^{-2} \cdot d^{-1}$ (days 224–233) and -59 mmol $C \cdot m^{-2} \cdot d^{-1}$ (days 233–236). The net mass of carbon lost was 1.7, 2.8, and 2.1 g $C \cdot m^{-2}$, respectively, for a total of 6.6 g $C \cdot m^{-2}$.

DIC in the epilimnion increased during the experiment by ~70 μmol/L. This corresponded to a net gain of 4.3 g C/m^2 over the duration of the experiment. In order to balance the carbon budget for the limnocorral, a source of carbon providing 4.3 g C/m^2 (DIC increase) + 6.6 g C/m^2 (gas exchange) = 10.9 g C/m^2 was necessary. The entrainment of carbon during the deepening of the epilimnion could have provided such a source. DIC and POC had a large gradient within the thermocline and average values for these quantities

TABLE 2. Activity measurements and specific activity calculations for DOC, DIC, and POC.

Day	$(^{14}C/C)_{DIC}$ dpm/μmol	$(^{14}C/C)_{DOC}$ dpm/μmol	$(^{14}C/C)_{POC}$ μmol	Σ¹⁴C dpm/L DIC	DOC	POC
220 A.M.	157.3	0.04	BACKGROUND	10851	+ 16	+ 0
220 P.M.	140.5	0.03	0.65	9837	+ 11	+ 40
221	122.0	0.09	0.75	8906	+ 43	+ 55
222	89.5	0.03	1.14	9142	+ 17	+ 81
224	62.0	0.12	1.27	6572	+ 49	+ 93
225	52.0	0.04	1.68	5930	+ 17	+ 129
227	45.8	0.01	1.76	5637	5	153
228	38.5	0.14	1.64	5040	+ 78	146
229	34.3	0.02	1.68	4666	+ 11	+ 153
231	26.7	0.05	2.26	3812	+ 32	+ 185
233	24.2	0.04	3.19	3637	+ 20	+ 233
234 A.M.	24.5	0.08	3.84	3699	+ 37	+ 261
234 P.M.	22.5	0.09	4.21	3417	38	+ 316
235	18.8	0.17	3.17	3027	+ 73	+ 263
236	17.3	0.08	3.28	2748	+ 37	+ 269
237	16.8	—	4.25	2649	+ —	327

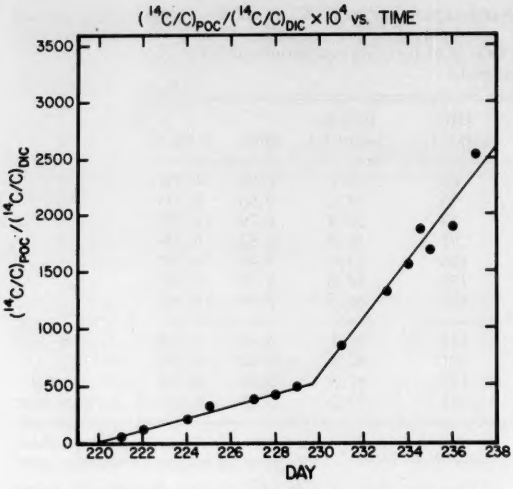

FIG. 3. Specific activity ratio of particulate organic carbon and dissolved inorganic carbon vs. time.

in the entrained thermocline waters were 400 and 2800 μg C/L, respectively. There was no significant DOC gradient. Entrainment of 1.1 m of thermocline water would have added 4.5 g C/m^2 of DIC and 2.1 g C/m^2 of POC over the duration of the experiment. Since there was no net change in POC levels over the course of the experiment (see Table 1), all POC above this level entrained during epilimnetic deepening must have been balanced by an equivalent loss of POC by oxidation or by settling. Sediment traps indicate that loss of POC by settling was 0.2 g C/m^2 over the 16 d of the experiment. Thus, additions of carbon through entrainment of thermocline waters amounted to 4.5 g C/m^2 (DIC entrainment) + 1.9 g C/m^2 (POC entrainment and oxidation minus sedimentation) = 6.4 g C/m^2, which accounts for 60% of the carbon fluxes into or out of the limnocorral epilimnion.

Comparison with Incubator Primary Production

In order to provide an independent estimate of daily integral primary production for comparison with incubator estimates, samples were taken directly from the limnocorral at sunrise and sunset on days 220 and 234. Values of 14.7 mg C·m^{-2}·d^{-1} for day 220 and 181 mg C·m^{-2}·d^{-1} for day 234 were determined for the limnocorral using equation 8 and the data in Table 2. Incubator estimates for these same 2 d were 12 and 156 mg C·m^{-2}·d^{-1}, respectively.

P*, or daily integral primary production minus nighttime or dark respiration (P–Rn), was calculated using the same equation (8) as that used for the calculation of P for days 220 and 234. The time scale for P* is not from dawn to dusk (as for P) but is from day-to-day extended over a period of 14 d. For days 220 through 234 the average P* is determined to be 29 mg C·m^{-2}·d^{-1}. An estimate of Rn becomes possible after examination of the relationship between

$$Z_e \frac{(^{14}C/C)_{POC}}{(^{14}C/C)_{DIC}} \text{ and time} \qquad \text{(see Fig. 3)}$$

since the slope = P* (see equation 8). Use of this plot is justified because POC concentrations were constant throughout the experiment and because the DO^{14}C contribution to $\Delta\Sigma^{14}C_{ORGANIC}$ could be ignored since DO^{14}C maintained a scattered but constant level just above the background and blank levels of the analytical technique. Figure 3 reveals a dramatic, abrupt shift in P* at day 230. By assuming a constant Rn:P ratio, it follows that $P_1 = 14.7$ mg C·m^{-2}·d^{-1} for days 220 to 230 and that $P_2 = 181$ mg C·m^{-2}.d^{-1} for days 230 to 234. An average value for Rn can then be calculated from the following equation

$$\Sigma \text{ net uptake of C} \cdot \text{m}^{-2} = 14 \text{ d} \times 29 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$$
$$= 10\text{d}\,(P_1 - xP_1) + 4\text{d}\,(P_2 - xP_2)$$

where xP = Rn. Thus, Rn = 0.53 P or 7.8 mg C·m^2·d^{-1} for days 220 to 234 and 96 mg C·m^{-2}·d^{-1} for days 230 to 234. Use of the incubator estimates for P, where $P_1 = 12$ mg C·m^{-2}·d^{-1} and $P_2 = 156$ mg C·m^{-2}·d^{-1}, gives Rn = 0.45 P and 5.5 and 70 mg C·m^{-2}·d^{-1} for their respective time periods. The assumption that Rn:P ratio is constant is not critical since the P_1 term is considerably smaller than the P_2 term in the equation above. If the value for daily integral primary production is close to net photosynthesis (Pn) and, if respiration during the day (Rd) = Rn, gross photosynthesis (Pg) may be calculated easily from the relationship

$$Pg = Pn + Rd = Pn + Rn$$

after corrections for the actual length of day (16 h) and night (8 h). Thus, for $P_2 = Pn = 181$ mg C·m^{-2}·d^{-1}, $Rd = 96\left(\frac{16}{8}\right) = 192$ mg C·m^{-2}·d^{-1}, and Pg = 181 + 192 = 373 mg C·m^{-2}·d^{-1}. Thus, Rd is 51% of gross photosynthesis. Similarly for $P_1 = 14.7$ g C·m^{-2}·d^{-1}, Rd = 15.6 g C·m^{-2}·d^{-1}, and Pg = 30 g C·m^{-2}·d^{-1}.

Total respiration was considerably higher. Since there was no net increase in POC throughout the experiment, an amount of POC equivalent to the extra POC entrained by deepening of the epilimnion must also have been respired. Oxidation or mineralization (m) of entrained POC mounted to an average of 186 mg C·m^{-2}·d^{-1}, with probable wide variation due to sporadic deepening of the epilimnion. Rn and Rd were low for days 220–230 but an order of magnitude higher for days 230–234. Total respiration could easily have ranged from 50 to 500 mg C·m^{-2}·d^{-1} increasing

throughout the experiment with an average value of $186 + \left(\frac{10}{14}\right)(7.8 + 15.6) + \left(\frac{4}{14}\right)(192 + 96) = 285$ mg $C \cdot m^{-2} \cdot d^{-1}$.

Measurements of respiration in the summer of 1976 (DeClercq et al. 1977) ranged from 340 to 422 mg $C \cdot m^{-2} \cdot d^{-1}$ for the same lake during a similar period of deepening thermocline in mid-August. Net primary production (PP_{net}) was, therefore, substantially negative. Since $PP_{net} = Rg - Rd - Rn - m = P^* - m$, then $PP_{net} = 29 - 186 = -157$ mg $C \cdot m^{-2} \cdot d^{-1}$.

Discussion

The potential usefulness of radiocarbon additions to large, enclosed volumes of water for the purpose of investigating the complex biogeochemical dynamics of carbon cycling has been clearly demonstrated with this experiment. Most significantly, the radiocarbon addition has provided the first opportunity to measure directly photosynthetic uptake by the ^{14}C technique, and to assess by direct comparison the accuracy of a ^{14}C "bottle" method. While directly measured values of primary productivity from the limnocorral are about 15% higher than the incubator estimates, the differences are within experimental error. Considering that there was an order of magnitude difference in primary productivity for the 2 d of comparison, the two methods give remarkably similar results and strongly support the validity of the incubator techniques used at ELA. Similar experiments have the potential for testing the validity of all the various ^{14}C bottle techniques used to measure primary productivity.

The ability to distinguish not only between nighttime and daylight respiration (Rn and Rd) and mineralization (m), but also to determine a value for net primary production (PP_{net}) was an unusual and significant feature of the limnocorral experiment, possible only through a combination of results from both the radiocarbon addition and the carbon budget. Certain assumptions should, however, be considered: because it has been shown that Rd may be less than Rn (Bunt 1965), Rd and therefore Pg must be considered maximum values. Furthermore, whether the ^{14}C technique measures gross or net photosynthesis or something in between has been debated at length. For incubations greater than 24 h the ^{14}C method should closely approximate Pn (Steemann-Nielsen 1977). While such a long incubation in bottles would not have been a reliable indicator of the natural environment, the direct measurement of ^{14}C uptake in the limnocorral provided the best available circumstance in which to measure net photosynthesis.

Uptake of ^{14}C during photosynthesis is often followed by excretion of labelled dissolved organics. Equal amounts of ^{14}C in both the DOC and POC pools were found throughout the four weeks of a radiocarbon addition to an oligotrophic lake (Hesslein et al. 1980). $DO^{14}C$ production in the limnocorral was negligible but should not be considered unusual. Highly variable rates of $DO^{14}C$ excretion ranging from 0 to over 50% of $PO^{14}C$ uptake have been reported and summarized (Thomas 1971); in addition, generally higher rates of DOC excretion have been reported for oligotrophic bodies of water.

The absence of data for both algal biomass and chlorophyll do not allow the dramatic increase in primary productivity beginning at day 230 to be termed an "autumn bloom." The classical explanation for autumn peaks of epilimnetic algal biomass and chlorophyll required the entrainment of nutrients from thermocline waters during the deepening of the mixed layer to stimulate algal growth. There is evidence, however, that autumn "blooms" can often result from the entrainment of previously produced chlorophyll and algal biomass (Fee 1976). Increased algal growth in the autumn should be correlated not only with peaks of algal biomass and chlorophyll but also with successive measurements of primary productivity by standard ^{14}C techniques during epilimnetic deepening. Such a dramatic increase in primary productivity was detected in the limnocorral by two different ^{14}C techniques. Because it occurred at the onset of epilimnetic deepening it may have been initiated by entrained nutrients and nutrients released by oxidation of entrained particulates. Since 66–86% of a lake's total chlorophyll may be contained in the hypolimnion with the majority of this in a large thermocline peak (Fee 1976), entrainment of this chlorophyll peak may swamp any increased chlorophyll production associated with the increased primary production of a real autumn bloom if the criteria for defining autumn blooms are restricted to measurements of algal biomass or chlorophyll. Increased primary productivity may also occur along with entrainment of previously produced algal biomass during epilimnetic deepening. Studies of algal biomass at the species level and, more importantly, measurement of the chlorophyll/production ratio are necessary in order to more fully interpret "autumn blooms."

The major source of error in the CO_2 gas exchange determination and, consequently, also in the carbon budget, is the calculation of aqueous CO_2 ($[CO_2]_L$) from DIC, pH, and temperature data. The pH sensitivity of the calculated $[CO_2]_L$ (or P_{CO_2}) values is considerable (Weiler 1975). Open-vessel pH measurements, as routinely performed at ELA, cannot prevent changes in DIC caused by CO_2 gas-exchange, particularly of oversaturated or undersaturated lake water. In waters of low buffer capacity postsampling CO_2 gas-exchange may substantially alter the original in situ pH. This is further complicated by the possibility of diurnal changes in P_{CO_2} due to biological activity. Such a diurnal pattern would make single daily pH and DIC measurements unrepresentative. Mass transfer coefficients (D/z) calculated for the limnocorral of 126, 56, and 100 cm $\cdot d^{-1}$ compare favor-

ably, but are somewhat higher, than those determined by the radon method (Hesslein et al. 1980) for 5 m diameter (mean = 58 cm·d^{-1}, range = 12–124 cm·d^{-1}) and 10 m diameter (73 cm·d^{-1}) limnocorrals. Moreover, the direction of the error in the mass balance for carbon could have resulted from an overestimation of gas flux.

An interesting feature of the gas exchange determinations was the clear correlation between periods of epilimnetic deepening (instability), higher average wind speeds, and higher values of the mass transfer coefficient. Mass transfer coefficients (D/z) for days 220–224 and 223–236, where $\Delta Z_e/\Delta t > 0.13$, were over 100 cm·d^{-1}. For the 9-d period in the middle of the experiment (224–233), where $\Delta Z_e/\Delta t < 0.02$, $D/z = 58$ cm·d^{-1}. Previous gas-exchange experiments using wind–wave tunnels have documented the dependence of the mass transfer coefficient on wind speed (Downing and Truesdale 1955; Kanwisher 1963; Hoover and Berkshire 1969; Liss 1973). Recent work has greatly extended the range of wind speed and convincingly demonstrated the linear dependence of the mass transfer coefficient on wind speeds >3 m/s (Broecker et al. 1978). The relationship in the region between wind speeds of 2–3 m/s, where capillary waves begin to develop, is nonlinear; our limnocorral results lie in this critical area and are close to those determined in wind–wave tunnels. In as much as free-stream velocities were measured both by Broecker et al. (1978) and at the ELA meteorological site, the consistency of the results suggest not only that laboratory simulations with wind–wave tunnels and large water tanks (Broecker et al. 1978) may have direct applicability in predicting mass transfer coefficients for lakes from wind speed measurements but also that limnocorral radiotracer experiments, with wind velocities carefully measured at the experimental site, will provide a useful tool to continue the effort to predict gas-exchange flux in a wide variety of natural water systems using easily measured parameters.

Acknowledgments

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Errors in Molybdenum Blue Methods for Determining Orthophosphate in Freshwater

M. P. STAINTON

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

STAINTON, M. P. 1980. Errors in molybdenum blue methods for determining orthophosphate in freshwater. Can. J. Fish. Aquat. Sci. 37: 472–478.

Whole lakewater was spiked with carrier free $^{32}PO_4$, filtered through a 0.45-μm membrane filter and the filtrate fractionated using G-25 Sephadex. Three molecular weight fractions incorporated ^{32}P labeling, one of which was colloidal. This labeled filtrate was treated with several variations of the molybdenum blue method for measuring orthophosphate followed by molecular weight fractionation. All variations in the molybdenum blue method caused a significant loss of phosphorus from the colloidal fraction. Phosphorus originating from the colloidal fraction tested as orthophosphate giving an overestimate of the true orthophosphate present.

Key words: orthophosphate, analytical errors, freshwater

STAINTON, M. P. 1980. Errors in molybdenum blue methods for determining orthophosphate in freshwater. Can. J. Fish. Aquat. Sci. 37: 472–478.

A de l'eau de lac entière, nous avons ajouté du $^{32}PO_4$ sans porteur, l'avons filtrée à travers un filtre à membrane de 0,45 μm et fractronné le filtrat à l'aide de G-25 Sephadex. Des fractions de trois poids moléculaires ont absorbé ^{32}P, dont l'une colloïdale. Le filtrat marqué est ensuite traité selon plusieurs variations de la méthode au bleu de molybdène de détermination d'orthophosphate, suivie d'un fractionnement par poids moléculaire. Toutes les variations de la méthode au bleu de molybdène causent une perte importante de phosphore de la fraction colloïdale. Le phosphore provenant de la fraction colloïdale passe à l'analyse comme orthophosphate, ce qui a pour effet de surestimer la quantité réelle d'orthophosphate présent.

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MANY limnological studies and freshwater management strategies are based on estimates of orthophosphate in freshwater. Essentially all orthophosphate data have been obtained using the molybdenum blue method in one or other of its modifications. However, in the past decade there has developed in the literature a divergence of opinion and hence some uncertainty as to whether or not the molybdenum blue method measures biologically utilizable orthophosphate.

The use of $^{32}PO_4$ tracer techniques (Kuenzler and Ketchum 1962; Jones and Spencer 1963; Rigler 1966, 1968; Chamberlain 1968) has revealed a discrepancy between the ^{32}P labeled compartment that organisms and ion exchange resins discriminate as orthophosphate and the compartment measured chemically as orthophosphate using molybdenum blue methodology. Chemical estimates are always higher than tracer bioassay estimates, often by one to two orders of magnitude.

On the contrary, nontracer bioassays based on disappearance of molybdate reactive phosphorus and cell growth response (Chamberlain and Shapiro 1969; Walton and Lee 1972) show no significant differences from molybdenum blue method estimates once corrected for arsenate interference.

Suspicion that molybdenum blue methods are capable of inducing acid hydrolysis of organic phosphorus has been voiced in numerous publications (Harvey 1948; Martin and Doty 1949). Olsen (1966) suggested the existence of a colloidal phosphorus fraction which hydrolyzed during chemical analysis and recommended its removal using ultrafiltration. Minear (1972), using a molecular sieve technique to fractionate lakewater, found significant concentrations of phosphorus in a molecular weight fraction greater than 50 000. Much of the material was DNA fragments.

Lean (1973) used a combination of ^{32}P labeling and molecular sieve fractionation to isolate three molecular weight fractions which incorporated ^{32}P labeling when $^{32}PO_4$ was added to whole lakewater. All three fractions isolated by Lean were biologically utilizable, but at greatly different rates. For example, the rate constant for $^{32}PO_4$ uptake by phytoplankton was two orders of magnitude greater than that for colloidal phosphorus.

Attempts to study phosphorus kinetics in the Experimental Lakes Area of northwestern Ontario (total dissolved phosphorus typically less than 10 μg/L) led to a comparison between ^{32}P bioassay estimates and

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TABLE 1. Methods studied, reaction conditions, and observed colloid degradation.

Method	Reductant	Acid strength	Acid exposure time	Colloid ^{32}P degradation (%)
Molybdenum blue phosphate methods				
Harvey (1948)	Stannous chloride	0.37 *N*	8.5 min	90
Murphy and Riley (1962)	Ascorbic acid	0.40 *N*	10 min	60
Chamberlain and Shapiro (1969)	Stannous chloride	0.20 *N*	6 s	80
Molybdenum blue methods with isobutanol extraction				
Stephens (1963)	Ascorbic acid	0.18 *N*	1 min	90
Shapiro et al. (1969)	Stannous chloride	0.15 *N*	30 s	90

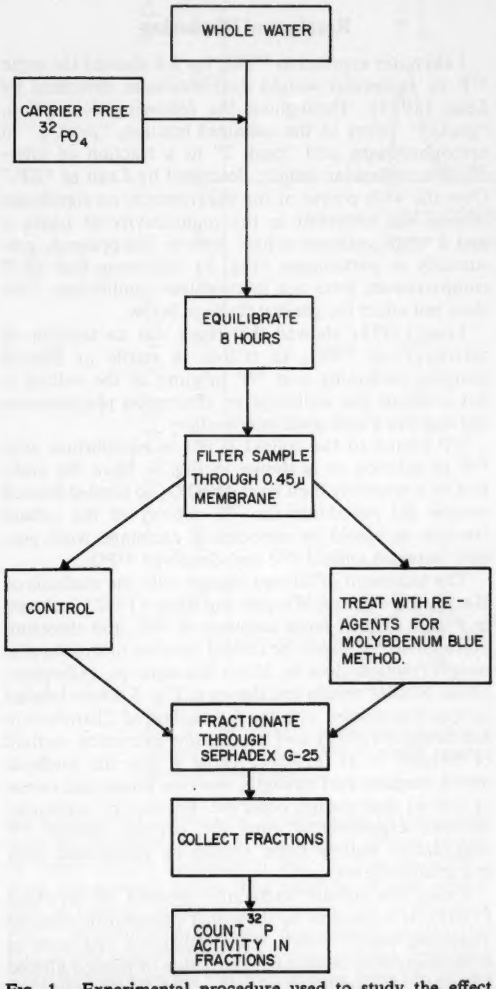

FIG. 1. Experimental procedure used to study the effect of molybdenum blue method reagents on colloidal phosphorus in freshwater.

molybdenum blue estimates which confirmed the one to two order of magnitude discrepancy observed by previous workers. Suspecting that the release of orthophosphate from colloidal phosphorus was the source of this error, we studied the effect of a variety of the more popular molybdenum blue methods on the colloidal phosphorus fraction.

Experimental Procedure

The experimental procedure used (Fig. 1) was based on techniques developed and used by Lean (1973). Whole lakewater containing 15 μg/L total dissolved phosphorus was collected from a depth of 1 m in Lake 227 of the Experimental Lakes Area (Schindler et al. 1973). Carrier-free $^{32}PO_4$ was added to give an activity of 50 000 dpm·mL^{-1} of lakewater. The inoculated sample was stirred at 25°C

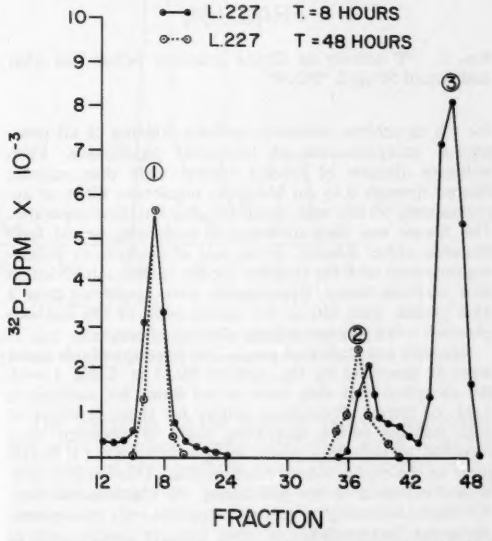

FIG. 2. ^{32}P activity of filtrate fractions after 8 and 48 h equilibrium prior to filtration. Molecular weight decreases with increasing fraction number. Peak 1 is the "colloidal phosphorus," >5000 in molecular weight. Peak 2 is equivalent to Lean's (1973) XP. Peak 3 is orthophosphate.

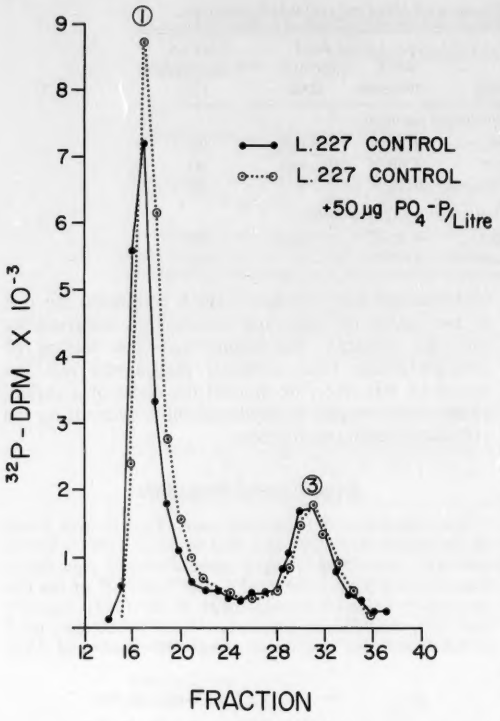

FIG. 3. ^{32}P activity of filtrate fractions before and after addition of 50 $\mu g/L$ $^{31}PO_4$-P.

for 8 h to achieve relatively uniform labeling of all phosphorus compartments of biological significance. Five-millilitre aliquots of labeled sample were then vacuum filtered through 0.45 μm Millipore membrane filters at approximately 50 mL/min, using all glass filtration apparatus. The filtrate was then subjected to molecular weight fractionation either directly, in the case of controls, or following treatment with the reagents for the various molybdenum blue methods tested. Experiments were conducted over a 48-h period, with filtrate for testing each of the methods obtained a few minutes before addition of reagents.

Reagents and analytical procedures for the methods tested were as described by the authors listed in Table 1 with the exception that they were scaled down for analysis of 5 mL of filtrate. Procedures calling for short exposure to acid reagents before measuring color development were modified to include a neutralization step with 1 N NaOH prior to molecular weight fractionation. This avoided prolonged exposure to low pH during the fractionation step. For methods employing solvent extraction only the aqueous phase was fractionated.

Molecular weight fractionation was achieved using a 2.5 × 35 cm column of G-25 "fine" Sephadex prepared according to the Pharmacia technical literature. Column eluant was 0.4% sodium chloride with 0.07% sodium azide in sterile distilled water and was pumped at 2 mL/min. Two-millilitre samples of filtrate were injected onto the column and collected in 4-mL fractions. A column void volume of 68 mL was measured using Blue Dextran 2000 while the orthophosphate retention volume of 184 mL was measured using $^{32}PO_4$. ^{32}P activity in the fractions was measured by Cerenkov counting. Measured activity was corrected for quenching, radioactive half-life, and dilution imposed by the chemical methods tested. A 2 mL · min^{-1} flow rate was used throughout (except Fig. 3).

An exception to the above is the data appearing in Fig. 3, where the void volume remained at 68 mL but orthophosphate retention volume was only 128 mL, and the flow rate was 4 mL · min^{-1}.

Results and Discussion

Lakewater exposed to $^{32}PO_4$ for 8 h showed the same ^{32}P vs. molecular weight distribution as described by Lean (1973). Throughout the following description, "peak 1" refers to the colloidal fraction, "peak 3" to orthophosphate, and "peak 2" to a fraction of intermediate molecular weight, described by Lean as "XP." Over the 48-h course of the experiments, no significant change was observed in the radioactivity of peaks 1 and 2 while orthophosphate activity disappeared, presumably to particulates (Fig. 2) indicating that all P compartments were not in complete equilibrium. This does not affect the general findings below.

Lean (1973) showed that there was no transfer of activity from $^{32}PO_4$ to colloid in sterile or filtered samples, indicating that ^{32}P labeling of the colloid is not a simple ion exchange or absorption phenomenon but requires a biological intermediary.

^{32}P bound to the colloid is not in equilibrium with ^{31}P in solution as is shown in Fig. 3. Here the addition of a relatively high level of $^{31}PO_4$ to labeled filtered sample did not dilute the ^{32}P activity of the colloid fraction as would be expected if exchange were possible between colloid ^{32}P and dissolved $^{32}PO_4$.

The treatment of labeled sample with the methods of Harvey (1948) and Murphy and Riley (1962) is shown in Fig. 4 to shift large amounts of ^{32}P, and therefore presumable ^{31}P, from the colloid fraction to a molecular weight fraction close to, if not the same as, orthophosphate. Similar results are shown in Fig. 5 where labeled sample was treated with the 6-s method of Chamberlain and Shapiro (1969) and the alkaline extraction method of Shapiro et al. (1969). Table 1 lists the methods tested, reagent acid strength, reaction times, and extent of colloid degradation observed. Because of variability between experimental runs the percent colloid ^{32}P degradation figures listed should be interpreted only in a qualitative way.

Using the solvent extraction method of Stephens (1963), it is possible to show that phosphorus released from the colloid reacts with molybdate and tests as orthophosphate. Isobutanol extraction of labeled filtered lakewater (no addition of acid molybdate reagents) is shown in Fig. 6 (upper panel) to have no effect on ^{32}P distribution or level in the aqueous phase. Colloid is

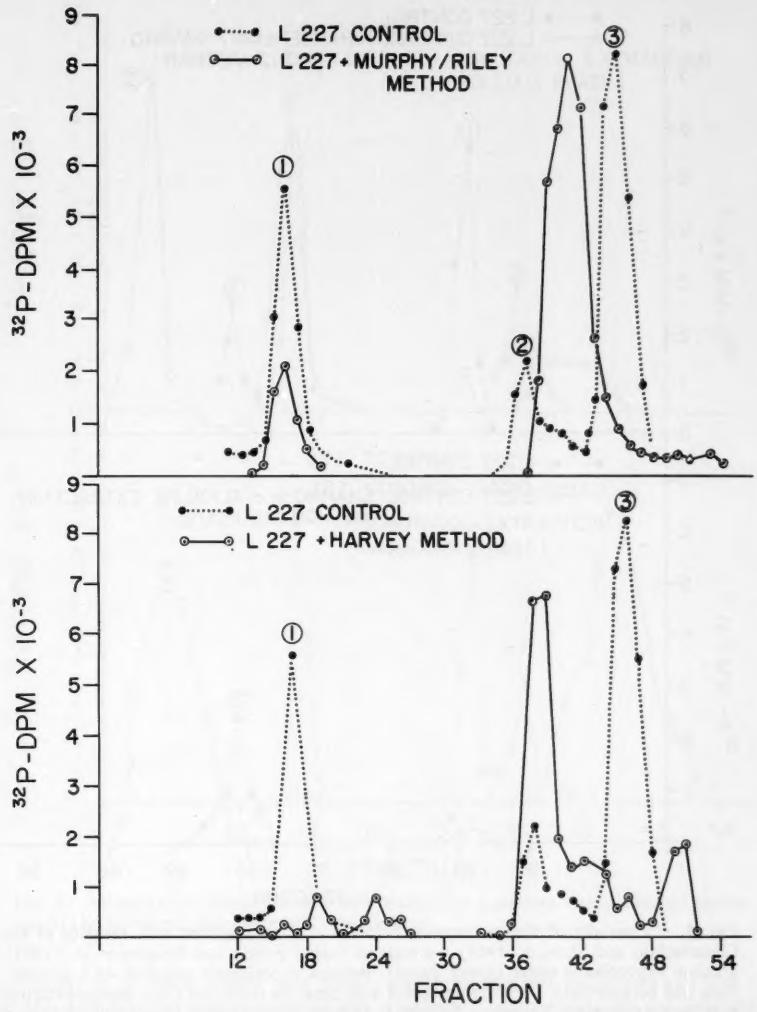

FIG. 4. ^{32}P activity of filtrate fractions before and after treatment with reagents of the Murphy and Riley (1962) single reagent method (upper panel) and Harvey's (1948) method (lower panel).

not extractable in isobutanol. Acidification of the sample prior to extraction is shown in Fig. 6 (lower panel) to cause colloid degradation but no loss of activity from the aqueous phase. The addition of acid molybdate to labeled sample prior to extraction is shown in Fig. 7 to induce both colloid degradation and extraction of ^{32}P activity from the aqueous phase indicating a reaction between the ^{32}P disappearing from the colloid and the molybdate yielding an extractable phosphomolybdenum complex.

The specificity of the reaction between molybdate and orthophosphate is well established. Since all of the molybdenum blue methods tested degraded part of the colloidal phosphorus fraction to orthophosphate, it is reasonable to deduce that phosphorus originally in the colloid reacted with molybdate and tested as orthophosphate.

The use of ^{32}P labeling followed by molecular weight fractionation permits a direct comparison between biological detection and chemical detection of some of

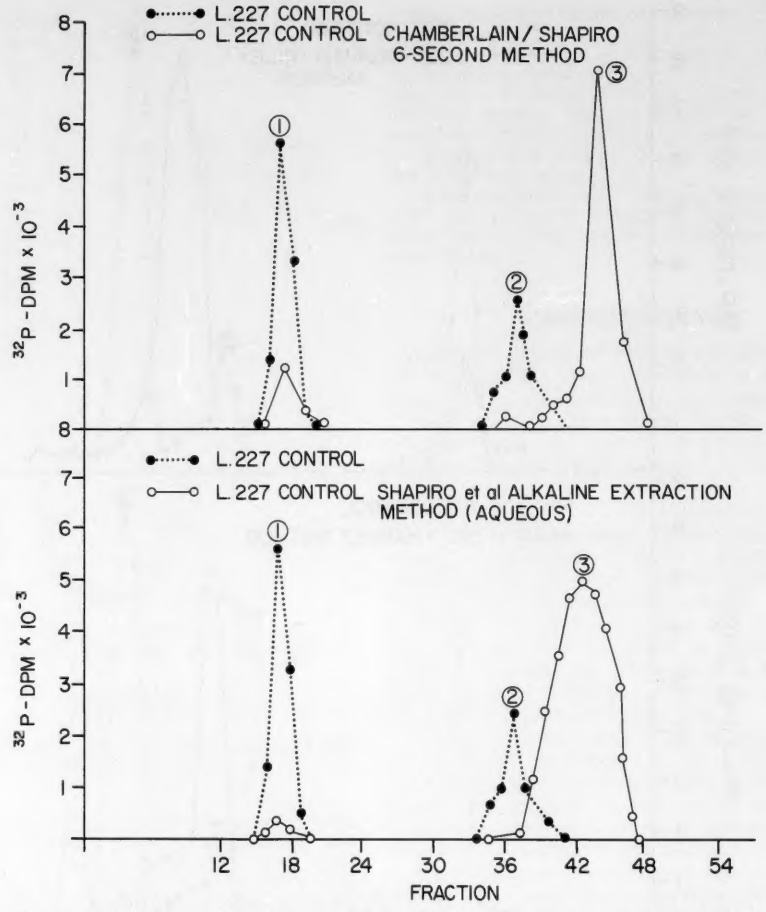

FIG. 5. ^{32}P activity of filtrate fractions before and after treatment with reagents of the Chamberlain and Shapiro (1969) 6-s method (upper panel) and Shapiro et al. (1969) alkaline extraction method (lower panel). Because experiments required 48 h to complete and because peak 3 activity decreased with time, we could not run a separate control with each experiment. Therefore, "controls" (having constant peak 1 and 2 activity but a steadily declining peak 3) in Fig. 4 to 6 are those controls fractioned nearest in time to the experimental fractionations. The behavior of "control" peak 3 is irrelevant to our main argument.

the components of the dissolved phosphorus pool. Lean (1973) showed that of the three ^{32}P fractions labeled and separated, only peak 3 (Fig. 2) tested biologically as orthophosphate (in terms of utilization rate constant). The findings reported above indicate that the tranfer of phosphorus from peak to peak during chemical analysis will cause overestimates of the compartment that is available to organisms as orthophosphate. These results appear to explain the differing conclusions reached by those using ^{32}P labeling and those using unlabeled bioassay techniques when testing the specificity of molybdenum blue methods.

Nontracer bioassay methods follow the depletion of the molybdate reactive phosphorus pool by organisms, usually over the course of hours if not days. Since Lean (1973) has shown that colloid phosphorus (peak 1)

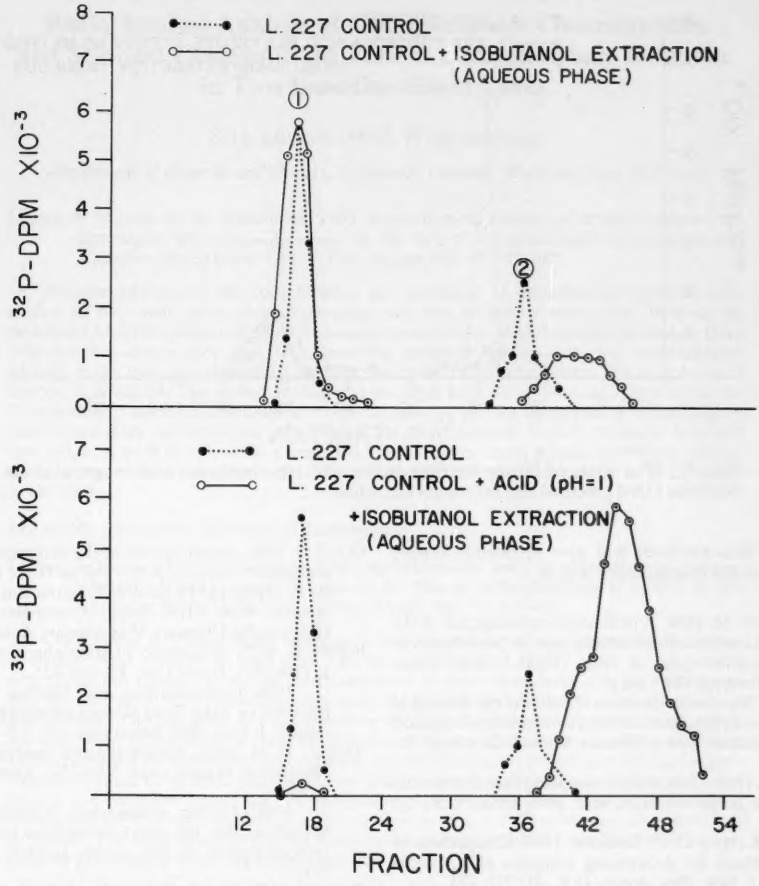

FIG. 6. ^{32}P activity of filtrate fractions before and after treatment with isobutanol (upper panel) and isobutanol plus dilute HCl (lower panel).

is utilizable (although at a much slower rate than orthophosphate) it is probable that organisms were able to take up part of peak 1 as well as peak 3 over such a long period. Estimates of available phosphorus using such techniques do not differ significantly from chemical estimates because they show the same lack of specificity.

On the other hand, the short-term $^{32}PO_4$ tracer techniques used by Lean and ion exchange techniques used by Rigler (1968) follow the response of organisms and ion exchange resins to peak 3 only and therefore, are at variance with chemical estimates.

The significance of the overestimation of orthophosphate with molybdenum blue methods depends on the ratio of colloidal phosphorus to orthophosphate. The water types most affected by overestimates are those containing low orthophosphate and high colloid P. Unfortunately, such waters are not amenable to methods which eliminate colloid interference (Westland and Boisclair 1974) because the orthophosphate present is below the detection limits of all known chemical techniques. Peters (1977, 1978), Rigler (1966), and Levine (1975) have successfully used radiotracer methods, but these are difficult and time consuming.

The significance of molybdenum blue overestimates is also dependent on the ultimate use of the data. If all that is required is an estimate of biologically utilizable dissolved phosphorus then chemical estimates may not be misleading. This remains to be tested. If, however, one requires estimates of orthophosphate for specific phosphorus kinetics studies, chemical estimates using

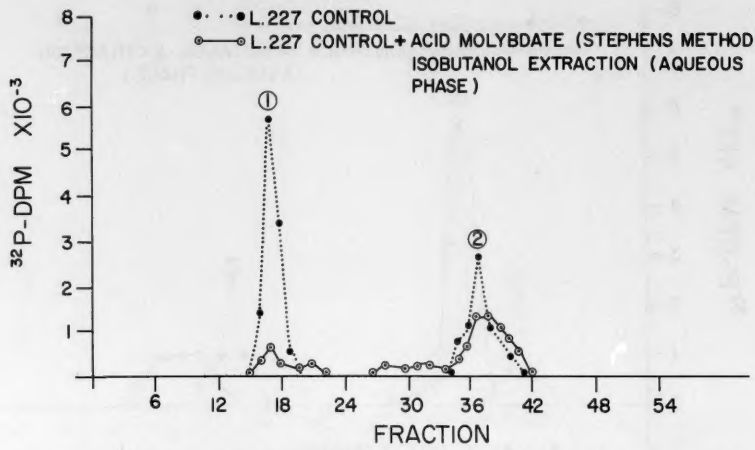

FIG. 7. ^{32}P activity of filtrate fractions before and after treatment with reagents of the Stephens (1963) method and isobutanol extraction.

molybdenum blue methods will give erroneous results in the waters of the Precambrian Shield.

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Radiochemical Analysis of Orthophosphate Concentrations and Seasonal Changes in the Flux of Orthophosphate to Seston in Two Canadian Shield Lakes

S. N. LEVINE AND D. W. SCHINDLER

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

LEVINE, S. N., AND D. W. SCHINDLER. 1980. Radiochemical analysis of orthophosphate concentrations and seasonal changes in the flux of orthophosphate to seston in two Canadian Shield lakes. Can. J. Fish. Aquat. Sci. 37: 479–487.

Seasonal changes in the concentration and dynamics of phosphate–phosphorus were studied in two small lakes, one oligotrophic and one artificially eutrophied. Because the molybdate blue phosphate technique frequently overestimates phosphate concentrations, three radiochemical assays were used. One, involving sephadex fractionation, was unsatisfactory because of the long period required for high molecular weight phosphorus fractions to reach isotopic equilibrium. The second method was unusable both for epilimnion waters within the Experimental Lakes Area, because of its low sensitivity, and for hypolimnion waters, due to interference from nonphosphate compounds. The third method, Rigler's bioassay, indicated that PO_4-P in both lakes seldom exceeded 0.1 $\mu g \cdot L^{-1}$, even under anoxic conditions. Organisms, and not mineral reactions, appeared to regulate the phosphate concentration at all depths in the lakes.

Key words: phosphorus dynamics, orthophosphate

LEVINE, S. N., AND D. W. SCHINDLER. 1980. Radiochemical analysis of orthophosphate concentrations and seasonal changes in the flux of orthophosphate to seston in two Canadian Shield lakes. Can. J. Fish. Aquat. Sci. 37: 479–487.

On a étudié les changements saisonniers de concentration et de dynamique de l'orthophosphate dans deux petits lacs, dont l'un est oligotrophe et l'autre a été artificiellement eutrophisé. Les concentrations de phosphate étant souvent surestimées avec la méthode au bleu de molybdate, trois essais radiochimiques ont été utilisés. Le premier, faisant appel à un fractionnement au sephadex, n'a pas donné de résultats satisfaisants à cause du temps requis par les fractions phosphore de poids moléculaire élevé pour atteindre un équilibre isotopique. Le deuxième n'a pu être utilisé, tant dans les eaux épilimnétiques de la région des lacs expérimentaux à cause de sa faible sensibilité, que dans les eaux hypolimnétiques à cause de l'interférence des composés autres que les phosphates. Le troisième, essai biologique de Rigler, indique que le PO_4-P dans les deux lacs dépasse rarement 0,1 $\mu g \cdot L^{-1}$, même dans des conditions anoxiques. Les organismes, et non les réactions minérales, semblent contrôler les concentrations de phosphate à toutes les profondeurs des lacs.

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TRADITIONALLY, the phosphorus cycle has been considered to be the best understood nutrient cycle in lakes. Early in this century it was discovered that the PO_4 ion was the form of phosphorus most readily available to algae. The early development of the molybdate blue method (Denignes 1921), which measured orthophosphate in $\mu g \cdot L^{-1}$ concentrations, became one of the most widely employed limnological techniques.

More recently, however, Downes and Paerl (1978) and Stainton (1979) have found that the molybdate blue method overestimates phosphate in lakes because the acid conditions under which the analysis is run cause hydrolysis of PO_4 from large molecules. Data from ion exchange (Jones and Spencer 1963; Rigler 1968), from Sephadex fractionations (Lean 1973; Stainton 1980), and from phosphorus uptake kinetics (Kuenzler and Ketchum 1962; Rigler 1966) have shown that only a small portion of soluble reactive phosphorus behaves as PO_4-P.

Because the molybdate blue method severely overestimates the PO_4-P concentrations of many lakes, published descriptions of seasonal and spatial changes in phosphate concentration based on the method are of doubtful validity. As a result, our understanding of the cycle of phosphorus, the element most likely to control aquatic productivity, is questionable. Unfortunately, alternative chemical methods for measuring phosphate have not been devised. Three radiochemical approaches have been suggested which appear to provide sufficient sensitivity. Although the application of these methods is tedious and requires considerable technical expertise,

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TABLE 1. Physical characterization of Lakes 227 and 302 S and phosphorus and chlorophyll data for the euphotic zone during the ice-free season.

Lake	Area (ha)	Z max (m)	$\bar{Z}$ (m)	Average weekly P loading ($\mu g \cdot L^{-1}$) Fertilizer	Natural	Mean PP ($\mu g \cdot L^{-1}$)	Mean TDP ($\mu g \cdot L^{-1}$)	Mean chlorophyll-*a* ($\mu g \cdot L^{-1}$)	Secchi (m) (midsummer)
227	5.0	10.0	4.4	11.4	2.6	42	11	50.0	0.6–2.2
302 S	10.9	10.6	5.5	—	0.9	6	3	16.5	3.1–5.1

we believed that a seasonal study of phosphate dynamics was essential to the understanding of the phosphorus cycle in lakes. For this reason, we undertook a study of the seasonal concentrations and dynamics of phosphate in two lakes (one oligotrophic and one artificially eutrophic) within the Experimental Lakes Area (ELA). The first year's results for the two epilimnions were reported earlier (Levine 1975). The present paper includes both epilimnion and hypolimnion data for the two lakes for 2 yr of study.

Description of the Lakes

Both lakes are small and well sheltered from the wind so that they are strongly stratified during the summer. Spring overturn in the two lakes is usually incomplete due to the rapid melting of ice and the onset of hot weather. During fall overturn, however, the lakes are normally in full circulation for 2–4 wk before freezing over.

Lake 227 has been fertilized with phosphate and nitrate since 1969, producing algal blooms characteristic of a eutrophic lake (Table 1) (Schindler et al. 1971, 1973; Schindler 1975). Lake 302 is separated at its narrows by a vinyl-impregnated nylon curtain. The hypolimnion of the North Basin receives fertilizer during the summer, but the South Basin is unfertilized and has remained oligotrophic (Table 1). Samples were collected only from the South Basin during this study.

Materials and Methods

Analytical Methods

The chemical methods used to measure total dissolved phosphorus (TDP) and particulate phosphorus were those of Stainton et al. (1974).

The first radiochemical approach entailed adding $^{32}PO_4$ to lake water and incubating the sample for several hours. The water was then filtered through a 0.45 μm Sartorius membrane and the $^{32}PO_4$ and other radioactive phosphorus fractions in the filtrate separated by Sephadex gel filtration (G25, fine resin). The water also was analyzed for total dissolved phosphorus concentration, [TDP]. The concentration of phosphate present in the sample was assumed to be:

$$[PO_4\text{-P}] \text{ in sample} = \frac{^{32}PO_4\text{-P in filtrate}}{\text{total } ^{32}P \text{ in filtrate}} \times [\text{TDP}] \text{ in sample.}$$

This technique had been previously used by Schindler et al. (1975). It is valid only if the specific activity of each phosphorus fraction, i.e. the proportion of phosphorus atoms which are radioactive, is the same as that for every other phosphorus fraction. This assumption was tested in the in situ column experiments described later.

The other two methods were based on the log-linear relationship between the rate of PO_4-P uptake by seston and the PO_4 concentration of lake water. Both methods involved the addition of $^{32}PO_4$ tracer to lake water followed by the calculation of the initial slope of the plot of ln radioactivity in the 0.45-μm filtrate fraction versus time. This slope is a close approximation of K, the $^{32}PO_4$-P uptake rate constant, and will be referred to as such in the remainder of this paper.[1] The first of these methods, earlier referred to as Schindler's bioassay (Levine 1976), entailed the preparation of a plot of K versus PO_4-P concentration for a standard sample (water from a eutrophic lake or an algal culture). A small amount of a sample of unknown PO_4 concentration was added to the standard along with a tracer amount of $^{32}PO_4$. The uptake constant rate which resulted was matched with the corresponding concentration value on the calibration curve and the PO_4-P concentration of the unknown was calculated.

The second kinetic technique employed was the radiobioassay developed by Rigler (1966). A range of PO_4 concentrations was created by adding unlabeled PO_4 to subsamples of lake water. $^{32}PO_4$ was injected along with the unlabeled PO_4 and the K for each PO_4 concentration determined (Fig. 1A). Uptake rate ($[PO_4\text{-P}] \times K$) was then plotted against PO_4-P concentration. The PO_4-P concentration used was that due to what was added plus an assumed original concentration. When the assumed value was too high the y-intercept of the rate-concentration graph was much greater than zero. As the estimate was reduced toward the true original concentration, the y-intercept approached zero (Fig. 1B).

All radioassays were done on 5-mL aliquots of filtrate, or filters suspended in distilled water. Samples were analyzed by Cerenkov counting (Brown 1971) using a Picker Liquimat 220 liquid scintillation counter.

Rigler bioassays were begun within 2 h of water collection. Unlabeled PO_4 was added to beakers containing 100–200 mL of lake water to create a range of added PO_4-P concentrations normally between 0.01 and 2.5 $\mu g \cdot L^{-1}$ in summer and between 0.5 and 50 $\mu g \cdot L^{-1}$ in winter. The unlabeled PO_4 solution and about 1 nCi (1 Ci = 37 GBq) of

[1]The slope would be equivalent to K if the exponential approached zero. The actual asymptote cannot be determined because, as will be demonstrated later in this paper, two-compartment analysis is not appropriate for data gathered more than a few minutes after $^{32}PO_4$ addition. During the summer, however, filtrate activity is normally less than 5% of the total radioactivity 24 h after tracer addition. Sephadex fractionation has indicated that less than 10% of this activity is $^{32}PO_4$ (Levine 1976).

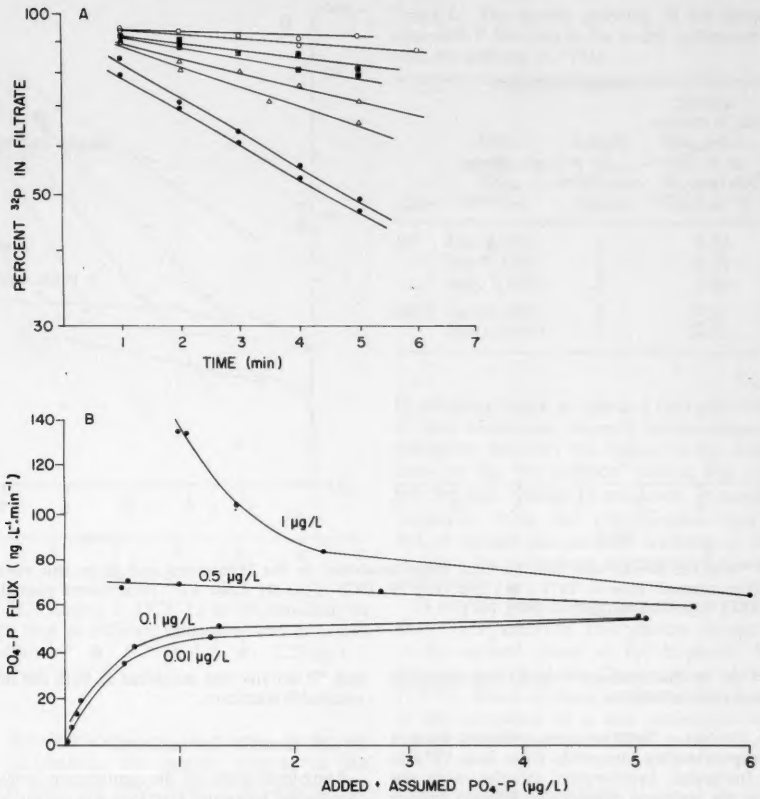


FIG. 1. An example of the plots involved in the Rigler bioassay. Lake 302 S, September 30, 1973. A) Ln% ^{32}P remaining in the 0.45 µm filtrate with time at different concentrations of added PO_4-P (no addition of unlabeled PO_4, ●; 0.5 µg·L^{-1}, △, 1.25 µg·L^{-1}, ■, 5 g·L^{-1}, ○). The lines shown are drawn by eye, but the K values used in the bioassay were determined by exponential regression. B) PO_4-P flux to seston vs. PO_4-P concentration. The assumed original concentration used for each plot is designated on the figure. More details are given in the text.

$^{32}PO_4$ were added simultaneously with Eppendorf pipettes. The samples were incubated under normal laboratory lighting (about 10 µE·m^{-1}·s^{-1}) at lake temperature and were stirred with a magnetic stirring rod while $^{32}PO_4$ uptake was followed.

LAKE SAMPLING

Surface water samples — Epilimnion samples were collected biweekly to monthly during the summers of 1973 and 1974. The samples were obtained by pooling water from two to three volumes of a 2 m long PVC tube which was lowered into the water, stoppered, withdrawn, and drained. The samples from Lake 227 were taken 2–7 d after the weekly fertilizations. During the winter, samples were collected from the two lakes on a monthly to bimonthly basis, using the same technique. At intervals, 5-mL aliquots of labeled lake water were withdrawn with a Cornwall syringe and passed through 0.45 µm Sartorius filters at a vacuum of 250 mm Hg. From the beginning of the study until July 1974, one aliquot was left unfiltered so that total and initial filtrate activity could be determined, but after that date all water was filtered and the filters were retained. Total activity then was calculated as the sum of the filter and filtrate activities.

The accuracy of the balances and flasks used in preparing solutions and of the Eppendorfs in delivering solutions limited the reliability of the uptake rate calculations to ±0.001 µg·L^{-1}·min^{-1}. Therefore, any assumed original concentration which produced a positive slope and a y-intercept of 0.001 µg·L^{-1}·min^{-1} on a rate-concentration graph was accepted as a maximum estimate of PO_4-P concentration. If the slope was negative when the y-intercept was 0.001 µg·L^{-1}·min^{-1}, the maximum PO_4-P concentra-

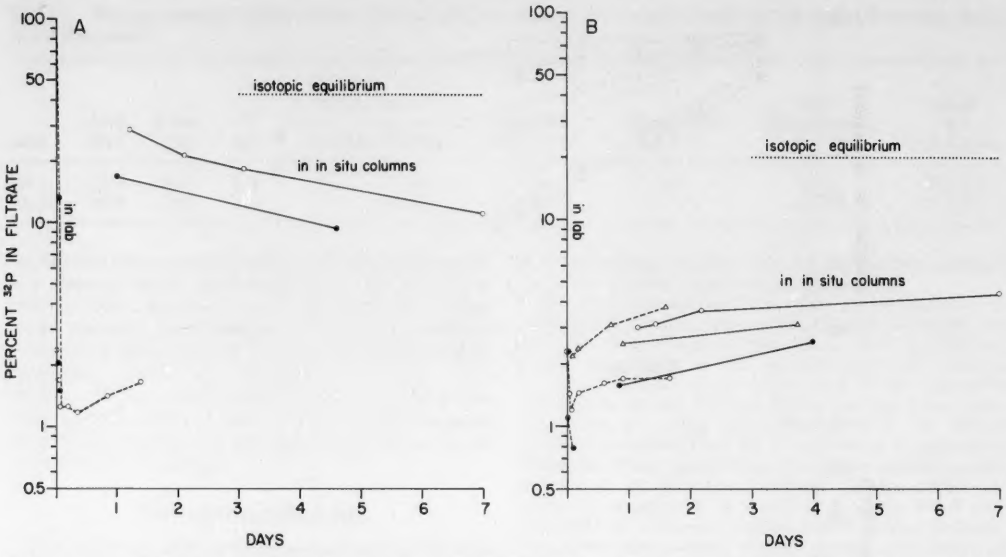


FIG. 2. Ln% ^{32}P in the filtrate with time for lake water incubated in the laboratory and in in situ columns. A) Lake 302 S. Incubations started: June 6, 1973 (●) and July 16, 1973 (○). B) Lake 227. Incubations started: June 7, 1973 (●), July 9, 1973 (○) and August 7, 1973 (△).

tion was taken to be the assumed original concentration which produced a slope of 0.000.

Deep water samples — Samples were collected from a depth of 9 m approximately bimonthly from June 1974 to March 1975. Integrated hypolimnion samples were not collected due to the technical difficulty of filling a syringe at more than one depth. A battery-powered pump and tygon tubing were used to draw water from the sampling depth. Fifty-millilitre glass syringes then were filled, inspected for bubbles, and, if bubble-free, closed with a three-way valve. The syringes were placed in fitted holes in a styrofoam box to prevent breakage and to keep the water near in situ temperature during transport. Although it was necessary for the syringes to be exposed to sunlight during filling, they were kept in darkness or very dim light (less than 0.1 $\mu E \cdot m^{-2} \cdot s^{-1}$) thereafter.

The radiobioassays for hypolimnion water had to be conducted without introducing oxygen into the samples. During June the ^{32}P solution was boiled to remove oxygen and then stored in glass syringes until needed. To avoid concentration, the unlabeled PO_4-P solution was not boiled. Less than 0.5 μg O_2 were introduced into a sample with this solution. Beginning in July, both PO_4 solutions were purged with nitrogen gas prior to use in hypolimnion radiobioassays. The solutions were injected into the mouth of the syringe with a fine needle.

After receiving $^{32}PO_4$ and unlabeled orthophosphate, the samples were shaken manually for 2 min and occasionally thereafter. At intervals, approximately 5 mL of water were expelled from each syringe. The water was quickly filtered and ^{32}P activity was measured in both the filtrate and filter-retainable fractions.

IN SITU COLUMN EXPERIMENTS

Long-term study of the equilibrium between ^{32}P and ^{31}P in different lakewater fractions was conducted using several polyethylene columns (3 m long and 27 cm in diameter) placed in the epilimnions of the lakes. Each column was suspended from the surface by a floating ring. Three columns were set up in Lake 227 and two in Lake 302 S. Each column received 2 mCi of carrier-free H_3 $^{32}PO_4$. Integrated samples were obtained from the column by lowering a PVC tube (2 m long and 3.25 cm in diameter), stoppering it and raising it quickly to the surface. Due to the rapid uptake of $^{32}PO_4$ by seston during the first few hours following injection, a subsample of epilimnion water was taken to the laboratory, where rapid mixing techniques allowed uptake to be studied more precisely during this initial period.

Results

IN SITU COLUMNS

As expected, $^{32}PO_4$ added to beakers in the laboratory and to in situ columns of epilimnion water was rapidly converted to sestonic ^{32}P (Fig. 2). A few hours after ^{32}P addition, the specific activity of the filter retainable fraction greatly exceeded that of the filtrate. Over the next few days, however, ^{32}P was slowly re-

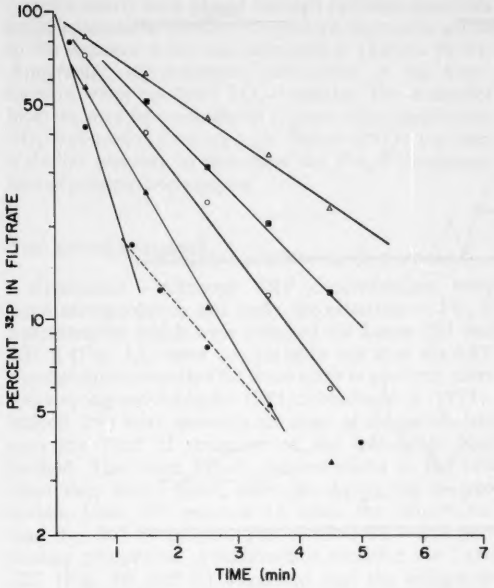


FIG. 3. Lake 227, August 7, 1973. Ln% ^{32}P remaining in the filtrate with time at different concentrations of added PO_4-P ("no addition," ●, 0.25 $\mu g \cdot L^{-1}$, ▲, 1.25 $\mu g \cdot L^{-1}$, ○, 5 $\mu g \cdot L^{-1}$, ■, and 10 $\mu g \cdot L^{-1}$, △).

turned to the filtrate phosphorus compartment. By the final day of incubation, the specific activity of the filtrate fraction was still only 8–23% of that of the filter retainable fraction (Table 2). The proportion of total ^{32}P in the filtrate fraction was 5–28% of what it would be at isotopic equilibrium, when all filtrate P would be labeled. A large fraction of filtrate phosphorus is therefore labeled only very slowly, if at all. Consequently, radiochemical methods which require that the distribution of ^{32}P be identical to that of ^{32}P in all phosphorus fractions, such as the sephadex method, could not be used to estimate PO_4 concentrations in our lakes.

Reliability of the Rigler Bioassay and Multi-Phasic Uptake

In general, the replicability of the rate constant for PO_4-P uptake, K, was very good (±10%) (Fig. 1A). Early in the project, however, apparent inconsistencies in the relationship between K and PO_4-P concentration were noted at added PO_4-P concentration less than 0.5 $\mu g \cdot L^{-1}$. The confusion proved to be due to an unanticipated, but persistent, shift in uptake rate during the first 1 or 2 min of $^{32}PO_4$-P uptake (Fig. 3).

TABLE 2. The specific activities of the filtrate P and filter retainable P fractions in the in situ epilimnion columns 4–7 d after the addition of $^{32}PO_4$.

Lake	Date of $^{32}PO_4$ addition	Length of incubation (days)	Specific activity of the filter retainable P on the final day (nCi·μg^{-1})	Specific activity of the filtrate P on the final day (nCi·μg^{-1})
227	June 7, 1973	4	7.58	0.592
	July 9, 1973	7	3.38	0.368
	Aug. 7, 1973	4	1.63	0.188
302 S	June 6, 1973	5	27.8	5.73
	July 16, 1973	7	34.3	7.73

If sampling began as late as 1 min after $^{32}PO_4$ addition, K was sometimes severely underestimated (note the difference between the slopes of the dotted and solid lines for the "no addition" plot in Fig. 3). The reason for the rate change is unknown. It might result from excretion, from the simultaneous operation of two PO_4-P uptake mechanisms working at different rates, or from the successive operation of a two-step uptake mechanism.

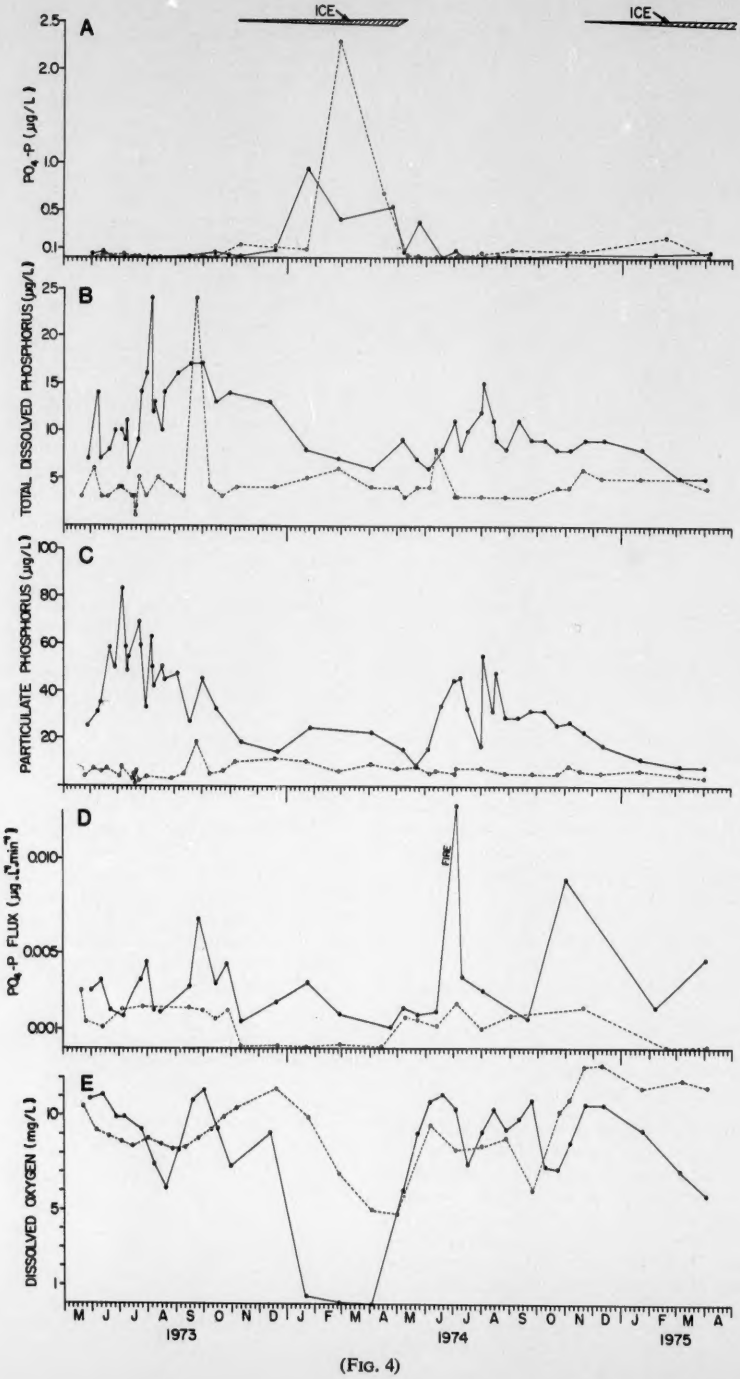
A second rate change sometimes occurred 5–60 min after $^{32}PO_4$ addition. This velocity change corresponded to the second phase of the bi-phasic PO_4-P uptake kinetics described by Chamberlain (1968) and Lean (1973). These authors attributed this break in velocity to the excretion of a low molecular weight organic phosphorus compound, "XP."

The Schindler Bioassay

The Schindler bioassay was not sensitive enough to estimate the orthophosphate concentration of ELA epilimnion water (Levine 1976). Concentration estimates for sediment interstitial water samples and for hypolimnion samples were obtained, but the values for the hypolimnion samples were consistently higher than the maximum values yielded by the Rigler bioassay during that summer (Table 3). Hypolimnion water

TABLE 3. Comparison of Schindler bioassay values for PO_4-P concentration at a depth of 9 m with the maximum concentrations measured by the Rigler bioassay during the summer of 1974.

Assay	Lake	Date	PO_4-P ($\mu g \cdot^{-1}$)L
Schindler's	227	Aug. 16, 1974	1.3
	227	Sept. 20, 1974	750.0
	302 S	Sept. 3, 1974	1.7
Rigler's	227	July 22, 1974	0.18
	302 S	June 21, 1974	0.084


ICE
ICE
A
PO_4-P (µg/L)
B
TOTAL DISSOLVED PHOSPHORUS (µg/L)
C
PARTICULATE PHOSPHORUS (µg/L)
D
PO_4-P FLUX (µg.L^{-1}min^{-1})
FIRE
E
DISSOLVED OXYGEN (mg/L)
M J J A S O N D J F M A M J J A S O N D J F M A
1973
1974
1975

(FIG. 4)

samples which were passed through hydrous zirconium oxide columns to remove PO_4 before they were added to the standard water also depressed K (Levine 1976). Apparently, nonphosphate compounds in the hypolimnion water inhibited PO_4-P uptake. The Schindler bioassay may be workable in regions where epilimnion PO_4-P concentrations are high. Peters (1977) has used a similar bioassay to determine the PO_4-P concentration of precipitation samples.

The Rigler Bioassays

Epilimnion — Although SRP concentrations were not measured during this study, the estimates of PO_4-P concentration which were obtained for Lakes 227 and 302 S (Fig. 4A) were considerably less than the SRP concentrations reported for these lakes in previous years (Armstrong and Schindler 1971; Schindler et al. 1973). Indeed, they were normally an order of magnitude less than the limit of detection of the molybdate blue method. The mean PO_4-P concentrations in the two lakes were nearly equal, although, during the ice-free season, Lake 227 received 15 times the phosphorus load that 302 S received. The elevated TDP and particulate phosphorus concentrations recorded for Lake 227 (Fig. 4B and C) suggested that the phosphate added to this lake each week was converted rapidly and almost entirely to particulate phosphorus and colloidal phosphorus.

In both lakes, the average flux of PO_4-P to seston during the ice-free season (Lake 227, 32 $\mu g \cdot L^{-1} \cdot wk^{-1}$; Lake 302 S, 17 $\mu g \cdot L^{-1} \cdot wk^{-1}$) (Fig. 4D) exceeded the phosphorus loading into the epilimnion (Table 1). The discrepancy between loading and flux indicated that phosphorus entering the epilimnion of Lake 302 S is recycled through seston an average of 17 times prior to sedimentation. In Lake 227, entering phosphorus is cycled twice through seston before sedimenting. In order to allow this, the tiny phosphate pool must turn over 5250 times in Lake 227 and 6000 times in Lake 302 S.

The highest PO_4-P concentrations were measured in late winter when dissolved oxygen concentrations were low (Fig. 4A and E). PO_4-P concentration and oxygen concentration, however, were not directly related. Lake 302 S boasted higher PO_4-P concentration than Lake 227 even though its dissolved oxygen concentrations were greater.

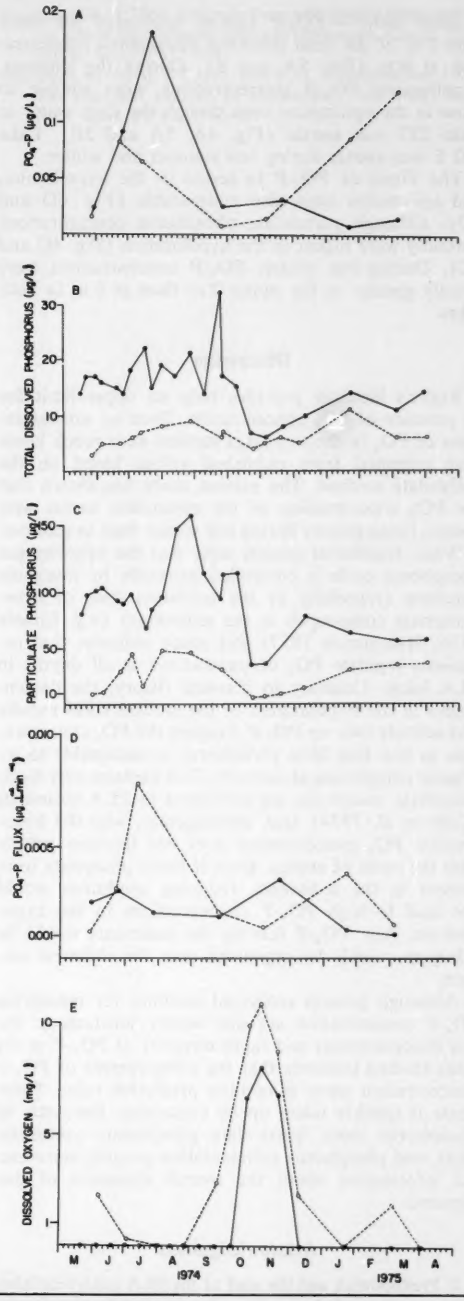


FIG. 5. Seasonal changes in A) PO_4-P concentration, B) total dissolved phosphorus concentration, C) particulate phosphorus concentration, D) the flux of PO_4-P to seston and E) dissolved oxygen concentration, at 9 m in lakes 227 (●) and 302 S (○). In error, the January samples were taken from 9.5 m rather than 9.0 m.

FIG. 4. (*Facing page*) Seasonal changes in A) PO_4-P concentration, B) total dissolved phosphorus concentration, C) particulate phosphorus concentration, D) the flux of PO_4-P to seston, and E) dissolved oxygen concentration, in the epilimnions of lakes 227 (●) and 302 S (○).

Deep water — PO_4-P seldom accounted for more than 1% of the total dissolved phosphorus concentration at 9 m (Fig. 5A and B). During the summer, hypolimnion PO_4-P concentrations were similar to those in the epilimnion even though the deep water in Lake 227 was anoxic (Fig. 4A, 5A and 5E). Lake 302 S was anoxic during late summer and winter.

The fluxes of PO_4-P to seston in the hypolimnion and epilimnion were also comparable (Fig. 4D and 5D), although particulate phosphorus concentrations normally were higher in the hypolimnion (Fig. 4C and 5C). During the winter, PO_4-P concentrations were usually greater in the upper 2 m than at 9 m in both lakes.

Discussion

Rigler's bioassay provides only an upper limit for its estimate of PO_4 concentration. Even so, concentrations of PO_4 in the two lakes studied were much lower than expected from published values based on the molybdate method. The present study has shown that the PO_4 concentration of the epilimnion varies with season, being greater during late winter than in summer.

While traditional models infer that the hypolimnion phosphorus cycle is controlled primarily by inorganic reactions (especially by the oxidation state of iron–phosphate compounds in the sediments) (e.g. Einsele 1936; Hutchinson 1957) this study indicates that organisms regulate PO_4 concentrations at all depths in ELA lakes. Contrary to classical theory, the decomposers in the hypolimnion of the studied lakes rapidly and actively take up PO_4-P, keeping the PO_4 concentration so low that little phosphorus is susceptible to inorganic complexing at overturn. This explains why ferric phosphate complexes are not found in ELA sediments (Coey et al. 1974) and, subsequently, why the hypolimnion PO_4 concentration does not increase greatly with the onset of anoxia. Even if ferric phosphate were present in the sediments, reducing conditions would not lead to high PO_4-P concentrations in the hypolimnion. Any PO_4-P leaving the sediments would be taken-up quickly by organisms near the sediment surface.

Although present analytical methods for measuring PO_4-P concentration are not wholly satisfactory, the low concentrations and rapid turnover of PO_4-P in the lakes studied indicates that the measurement of PO_4-P concentration alone is of little predictive value. Phosphate is quickly taken up by organisms. Estimates of phosphorus input, particulate phosphorus concentrations, and phosphorus sedimentation provide more useful information about the overall dynamics of this element.

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Comparison of the Nitrogen-15 Uptake and Acetylene Reduction Methods for Estimating the Rates of Nitrogen Fixation by Freshwater Blue-Green Algae

B. M. GRAHAM[1]

304 N 41st Street, Seattle, Washington 98103, USA

R. D. HAMILTON

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

AND N. E. R. CAMPBELL

Department of Microbiology, University of Manitoba, Winnipeg, Man. R3T 2N6

GRAHAM, B. M., R. D. HAMILTON, AND N. E. R. CAMPBELL. 1980. Comparison of the nitrogen-15 uptake and acetylene reduction methods for estimating the rates of nitrogen fixation by freshwater blue-green algae. Can. J. Fish. Aquat. Sci. 37: 488–493.

The relationship of acetylene reduction to nitrogen-15 uptake was investigated using blue-green algal populations in three lakes in the Experimental Lakes Area, northwestern Ontario. Nitrogen fixation rates, as estimated with both techniques, were compared and acetylene to nitrogen ratios determined. Lake ratios ranged from 6.3 to 9.1 moles of acetylene reduced per mole of nitrogen fixed varying from sample to sample and also with the method of calculation. Explanations of the discrepancies between theoretical and empirical ratios are discussed; these include hypotheses of excretion of assimilated nitrogen-15 labeled material and interference from nitrogenase-mediated hydrogen production.

Key words: acetylene reduction, nitrogen-15 uptake, nitrogen fixation, C_2H_2/N_2 ratio determination

GRAHAM, B. M., R. D. HAMILTON, AND N. E. R. CAMPBELL. 1980. Comparison of the nitrogen-15 uptake and acetylene reduction methods for estimating the rates of nitrogen fixation by freshwater blue-green algae. Can. J. Fish. Aquat. Sci. 37: 488–493.

Les auteurs ont étudié la relation entre la réduction de l'acétylène et l'absorption d'azote-15, utilisant les populations d'algues bleu-vert dans trois lacs de la région des lacs expérimentaux du nord-ouest de l'Ontario. Les taux de fixation de l'azote, estimés selon les deux méthodes, ont été comparés, et les rapports acétylène:azote déterminés. Ces derniers dans les lacs se situent entre 6,3 et 9,1 moles d'acétylène réduit par mole d'azote fixé, avec variation d'un échantillon à l'autre et aussi d'après méthode de calcul. On analyse les explications des différences entre les rapports théoriques et empiriques; parmi celles-ci, on compte hypothèses d'excrétion de matériel assimilé marqué à l'azote-15 et interférence par production d'hydrogène par intervention de la nitrogénase.

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USE of the acetylene reduction technique as an alternative to the direct estimation procedures utilizing nitrogen-14 and nitrogen-15 has led to a rapid increase in the number of studies estimating in situ aquatic nitrogen fixation (for example Stewart et al. 1967; Flett et al. 1976; Burris and Peterson 1976). This assay is particularly attractive to investigators because of its sensitivity, speed, easy adaptability to field studies, and economy (Hardy et al. 1968; Stewart et al. 1967). Routine use of nitrogen-15 techniques (Nees et al. 1962; Stewart 1967; Keeney and Tedesco 1973; Flett 1977), while theoretically more appropriate, requires major equipment investments (a mass spectrometer or emission spectrometer), longer analysis time, and the use of more cumbersome techniques.

Nitrogen fixation rates are usually estimated from acetylene reduction rate data by the use of a theoretical conversion factor of three based upon the chemical reaction stoichiometry.

$$N_2 + 3\,H_2 \rightleftharpoons 2\,NH_3$$

$$3\,C_2H_2 + 3\,H_2 \rightleftharpoons 3\,C_2H_4$$

[1]Requests for reprints should be sent to B. M. (Graham) Quay, Department of Oceanography WB-10, University of Washington, Seattle, WA 98195, USA.

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However, previous comparisons between acetylene reduction and nitrogen-15 uptake rates demonstrated an acetylene to nitrogen ratio ranging from 1.7 to 8.6 moles of acetylene reduced per mole of nitrogen fixed for various nitrogen fixing populations (Hardy et al. 1973). These variations from the theoretical ratio may be due to a number of factors which will be discussed later. The fact that the variations exist is the key issue, for the very utility of the acetylene reduction assay becomes suspect.

Based on acetylene reduction estimates and the assumption of a 3:1 ratio, fixation of atmospheric nitrogen appeared to contribute substantially to the nitrogen income of three artificially eutrophied lakes in the Experimental Lakes Area (Flett 1977; Graham 1978). Assessment of the error associated with the use of the acetylene reduction technique was therefore critical for calculations of accurate nitrogen budgets for these lakes.

Materials and Methods

Calibration of the acetylene reduction method with the nitrogen-15 uptake method was undertaken using samples from three artificially fertilized lakes in the Experimental Lakes Area, northwestern Ontario (Johnson and Vallentyne 1971), Lake 226 NE, Lake 227, and Lake 304. During 1976 and 1977, when this study was made, the fertilizer weight ratios for Lakes 226 NE, 227, and 304, respectively, were 10:5:1 (C:N:P), 5:1 (N:P), and 10:1 (N:P) (Schindler 1977). At various times throughout the summer sampling season these lakes supported blue-green algal populations (predominantly *Anabaena* and *Aphanizomenon* species) capable of maintaining nitrogen fixation rates sufficient for rate comparison studies. Blue-green algal blooms were most pronounced in Lake 227 in 1976 and 1977. Low levels of blue-green algal nitrogen fixation were apparent in Lake 226 NE and Lake 304 late in the 1976 and 1977 sampling seasons.

Sampling for nitrogen fixation determinations in all three lakes in 1977 was coordinated with knowledge of impending peaks in primary production (J. Shearer and D. DeClercq unpublished data) and the appearance of blue-green algae as one of the dominant phytoplankton groups (D. Findlay unpublished data). Because primary production peaks reflected contribution by many algal groups, only one of which was the blue-green algae, primary production peaks were not always coincident with nitrogen fixation peaks.

Moderate sample concentration was necessary early in the sampling season due to sparse blue-green algal populations and low nitrogen fixation levels which would otherwise have prevented adequate comparison of methods. A 10-μm mesh phytoplankton net hauled through the 0–2.5 m region of the lakes yielded concentrated algal samples. Four to eight hauls drained to 100–200 mL were combined in a 1-L container from which 30-mL subsamples could be withdrawn for acetylene reduction and nitrogen-15 uptake assays. Comparison of blue-green algal cell counts, chlorophyll concentration, nitrogen weight, and nitrogen fixation rate estimates for concentrated and unconcentrated samples, collected on the same sampling date, indicated an approximate concentration factor of 10.

Concentration of late summer blue-green algal populations in Lake 227 in 1976 and 1977 was unnecessary. Instead water samples were pumped directly from specific depths (0, 1, 2, and 3 m). However, the volume of unconcentrated sample prepared for nitrogen-15 uptake incubations was increased from 30 to 50 mL to ensure that sufficient nitrogen was available for nitrogen-15 analysis.

Sample preparation, incubation, analysis, and rate calculation procedures for both acetylene reduction and nitrogen-15 uptake assays followed those described in detail by Flett et al. (1976), Flett (1977), and Graham (1978). Sample filtrates, collected following filtration of nitrogen-15 uptake samples, were recovered and stored at 4°C for subsequent investigations of nitrogen-15 excretion. Treatment of filtrate samples was also described by Graham (1978). Briefly, aliquots of unconcentrated filtrates and filtrates concentrated by roto-evaporation were dropped by syringe onto Reeve Angel 984H filters. When dry, filters were analyzed for nitrogen-15 enrichment utilizing the same vacuum rack–nitrogen-15 analyzer system required for analysis of particulate fraction nitrogen-15 content.

Two methods of calculation of acetylene to nitrogen ratios from fixation rate data were used, mean determination and linear regression analysis. A simple mean ratio was calculated from acetylene reduction and nitrogen-15 uptake rates accumulated in 1976 and 1977 for each lake. Estimation of the acetylene to nitrogen ratio by linear regression analysis required assignment of acetylene reduction rate data or nitrogen fixation rate data as dependent or independent variables. Because nitrogen-15 uptake estimation was characterized by a greater analytical error (Graham 1978), it was designated the dependent variable in the regression. Therefore, the reciprocal of the slope of the linear equation ($y = mx + b$) characterizing the relationship of nitrogen-15 uptake (y) to acetylene reduction (x) represents an estimate of the acetylene to nitrogen ratio. Linear regression analyses were performed using the individual lake data sets and the complete data set (Graham 1978).

Results and Discussion

None of the lakes examined exhibited reduction rates exceeding 10 μg ethylene produced$\cdot L^{-1}\cdot h^{-1}$ or 2 μg nitrogen fixed$\cdot L^{-1}\cdot h^{-1}$ (Table 1). Maximum rates for concentrated lake samples were of the same order as acetylene reduction rates measured for unconcentrated Lake 227 samples assayed during the course of the late summer *Anabaena* bloom. From this comparison it would appear that net haul concentration procedures described earlier did not significantly affect rate determinations. Samples with very low nitrogen fixation activity exhibited greater variability with respect to ratios calculated.

Acetylene to Nitrogen Ratio Estimation

Both linear regression and mean determination methods of acetylene to nitrogen ratio estimation were utilized here to facilitate comparison of ELA lake ratios with previously estimated values and to test the linearity of the acetylene to nitrogen relationship over the range of nitrogen fixation rates measured. Ratios calculated by simple mean determination ranged from 6.3 to 9.1 moles of acetylene reduced per mole of nitro-

TABLE 1. Example $^{15}N_2$ uptake rate and acetylene reduction rate estimates accumulated for Lake 226 NE and Lake 227 during the summers of 1976 and 1977. Rate percentages and C_2H_2:N_2 ratios are calculated directly.

Date	Sample description		$^{15}N_2$ Rate[d] ($\mu g\ N_2 \cdot L^{-1} \cdot h^{-1}$)	C_2H_2 Rate[a,d] ($\mu g\ C_2H_4 \cdot L^{-1} \cdot h^{-1}$)	$\frac{^{15}N_2\ Rate}{N_2(C_2H_2)\ Rate^b}$	$\frac{C_2H_2}{N_2}$
Aug. 30, 1976	227[c]	0 m	0.174	1.239	42.1	7.1
		1 m	0.169	1.281	39.6	7.6
		2 m	0.121	1.035	35.1	8.6
June 22, 1977	304	0 m	0.843	6.150	41.1	7.3
		1 m	0.508	4.617	33.0	9.1
	304[c]	0 m	0.012	0.105	34.3	8.9
July 21, 1977	226NE	0 m	0.324	2.447	39.7	7.6
		1 m	0.345	2.447	42.3	7.1
	226NE[c]	0 m	0.035	0.267	39.3	7.6
Aug. 26, 1977	227[c]	0 m	0.860	5.738	45.0	6.7
		1 m	0.739	5.279	42.0	7.1
		2 m	0.346	3.428	30.3	9.9
		3 m	0.120	1.391	25.9	11.6

[a]C_2H_2 Rate — Acetylene reduction rate not converted to nitrogen fixation rate.
[b]$N_2(C_2H_2)$ Rate — Acetylene reduction rate converted to nitrogen fixation rate by assuming the theoretical C_2H_2:N_2 ratio of 3.
[c]Unconcentrated samples.
[d]Rate listed is the mean of duplicate samples.

gen fixed for the individual lakes (Table 2). Ratios estimated by linear regression analysis ranged from 7.2 to 8.8 (Table 2).

The similarity of acetylene to nitrogen ratios obtained for Lake 226 NE, Lake 227, and Lake 304 in 1976 and 1977 prompted determination of an overall ratio characterizing all lakes studied (Table 2, Fig. 1). Acetylene reduction and nitrogen-15 uptake data collected independently but analyzed similarly for Lake 226 NE and Lake 227 (Flett et al. 1980) yielded ratios of the same magnitude as those obtained in this study (Table 3); only two values fell outside the 95% confidence interval calculated for the overall lake ratio. Both sets of values are larger than average ratios calculated for Lake Mendota samples (4.8, Peterson and Burris 1976) and Bay of Quinté enclosures (5.7, Lean et al. 1976). This comparison suggests that both the specific lake examined as well as the particular analytical procedures used to measure nitrogen fixation rates influence the magnitude of the ratios.

To compare the two methods of ratio estimation the standard error of the mean and the error of the regression slope (i.e. the reciprocal ratio) were calculated (Table 2). Only one lake (Lake 304) shows agreement between the two ratio determinations within the error of the calculations. However, the overall lake data set yields a similar ratio independent of the method of ratio calculation. This agreement of mean and regression ratios for the overall lake data set and the zero intercept determined by the regression procedure for this data set (Table 2) suggest that a linear relationship exists between acetylene reduction rates and nitrogen-15 uptake rates over the range of rates measured in this study. The linearity of this relationship should be reexamined if a wider range of nitrogen fixation rates is sampled.

TABLE 2. C_2H_2:N_2 molar ratios estimated by mean and linear regression analyses. Ratios are calculated independently for each system prior to determination of overall ratios for the lake data.

System	Sample size	Mean ratio ± error	Regression ratio ± error	Regression intercept ± error	Correlation coefficient
L226–227 (1976)	21	6.6±0.2	8.8±1.3	0.02±0.01	0.83
L304	19	6.3±0.2	7.7±0.6	0.06±0.04	0.96
L226	17	8.2±0.8	8.3±1.0	0.01±0.02	0.90
L227	26	9.1±0.4	7.2±0.6	−0.04±0.04	0.92
All lakes	83	7.6±0.1	7.5±0.2	0.01±0.01	0.96

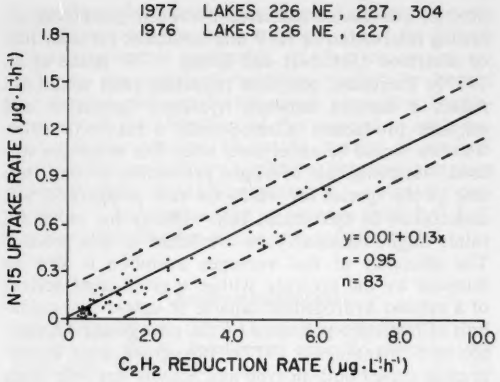

FIG. 1. A plot of the linear regression analysis of acetylene reduction rate and nitrogen-15 uptake rate data accumulated for Lake 226 NE, Lake 227, and Lake 304 in 1976 and 1977. The regression equation, correlation coefficient (r) and sample size (n) are provided. Dotted lines indicate the 95% confidence interval calculated assuming the applicability of a Student-t distribution.

EXPLANATION FOR THE DISCREPANCY BETWEEN THEORETICAL AND EMPIRICAL RATIOS

More than one hypothesis has been forwarded to account for the discrepancy between the theoretically derived and empirically measured acetylene to nitrogen ratios (Bergersen 1970; Lean et al. 1976; Walsby and Fogg 1975; Schubert and Evans 1976). The two most promising explanations include excretion of assimilated label (^{15}N) which is not detected during ^{15}N-analysis of the particulate fraction, and involvement of hydrogen as a competitor with nitrogen for necessary reductant and energy resources thereby reducing ^{15}N uptake estimates.

Fogg (1966), Walsby (1974), Walsby and Fogg (1975), and Jones and Stewart (1969) contributed evidence demonstrating that nitrogenous material was excreted from actively growing as well as unhealthy blue-green algal cultures. The most frequently observed extra-cellular products were amino acids and small peptides, predominantly serine, threonine, and basic amino acids (Fogg 1966; Jones and Stewart 1969).

In this study, where atom percent enrichment of nitrogen-15 in the particulate fraction was used to reflect the quantity of nitrogen fixation per unit time, loss of nitrogen-15-labeled product from the particulate fraction would have resulted in the high acetylene to nitrogen ratios found. Nitrogen-15 enrichment in the particulate fraction would then represent only assimilated nitrogen recoverable on the filter, rather than the total nitrogen fixed during the incubation interval. The acetylene reduction assay, requiring no sample filtration, would presumably estimate the total nitrogen fixed.

Two considerations, one empirical and one based upon a turnover time estimate for cell nitrogen, prevented acceptance of the excretion hypothesis as an adequate explanation of the difference between the ratios measured in this investigation and theoretical ratios. The empirical approach involved analyses of sample filtrate ^{15}N content. When labeled particulate material yielded nitrogen fixation rates lower than acetylene reduction-derived estimates, it was hypothesized that filtrates collected simultaneously should account for some of the missing isotope. Filtrates collected from samples incubated 2–3 h exhibited a mean ^{15}N enrichment (sample atom percent minus natural abundance) of 0.005 atom percent (Table 4), which was less than the measured precision of the natural abundance determination (0.397 at. % ± 0.006 at. %). Therefore, on the basis of these filtrate analyses no statistically significant ^{15}N enrichment of filtrate nitrogen was apparent. The absence of nitrogen-15 label in the filtrate would also eliminate the possibility of label loss as a consequence of cell breakage on filtration.

In addition, the calculation of an approximate turnover time of the incorporated nitrogen, patterned on a

TABLE 3. Acetylene reduction and nitrogen-15 uptake rate data collected for Lake 226 NE and Lake 227 in 1974 and 1975 (Flett et al. 1980). Associated C_2H_2:N_2 ratios are calculated.

Date	Lake	C_2H_2 Rate ($\mu g\ C_2H_4 \cdot L^{-1} \cdot h^{-1}$)	$^{15}N_2$ Rate ($\mu g\ N_2 \cdot L^{-1} \cdot h^{-1}$)	$\frac{C_2H_2}{N_2}$
Sept. 04, 1974	226 NE	6.06	0.51	11.9
Sept. 04, 1975	226 NE	0.78	0.12	6.4
Sept. 12, 1975	226 NE	1.72	0.19	8.9
		1.73	0.17	10.5
		1.30	0.14	9.1
		0.97	0.04	6.9
Sept. 18, 1975	226 NE	0.74	0.11	6.9
Sept. 24, 1975	226 NE	0.35	0.05	7.4
July 21, 1975	227	2.97	1.40	2.1
Sept. 18, 1975	227	0.72	0.09	8.2
Sept. 25, 1975	227	0.29	0.05	6.2

TABLE 4. Analyses of selected filtrate samples for ^{15}N atom percent enrichment above background and nitrogen weight. Filtrates receiving preliminary roto-evaporator concentration are noted (conc.).

System	Date	Atom % difference	N weight (mg/L)	Sample type[a]
L.304	June 22, 1977	0.002	16.6	1 mL unconc.
L.304	June 22, 1977	0.000	9.8	1.4 mL conc.
L.226 NE	July 06, 1977	0.006	28.7	1 mL unconc.
L.304	July 09, 1977	0.003	11.3	1 mL unconc.
L.226 NE	July 15, 1977	0.007	16.1	1 mL unconc.
L.227	July 27, 1977	0.016	0.2	1 mL unconc.
L.227	Aug. 24, 1977	0.000	7.6	1 mL unconc.

[a]Differentiation between filtrates that were concentrated and those that were not.

similar calculation described by Walsby and Fogg (1975) did not support the excretion hypothesis. The turnover time estimated for a representative unconcentrated Lake 227 sample using the equation

$$\text{Turnover time} = \frac{\text{(mass of particulate nitrogen/litre)}}{\text{(nitrogen fixation rate/litre)}}$$

was approximately seventeen days, which greatly exceeded the maximum incubation interval employed. Walsby's estimate of the turnover time for nitrogen assimilated by *Anabaena cylindrica* Lemm. cells was on the order of 9 d (Walsby and Fogg 1975). Therefore loss of labeled assimilation products during the short-term incubations of 2–3 h utilized in this investigation would not account for the discrepancy observed between nitrogen-15 uptake rates and acetylene reduction estimates.

A second hypothesis advanced to explain the discrepancy between the theoretical and empirical acetylene to nitrogen ratios proposes a competition between nitrogen and hydrogen which affects the magnitude of measured nitrogen fixation rates. Empirical evidence for hydrogen production in blue-green algae and descriptions of the process and its responses to various environmental manipulations has accumulated recently (Jones and Bishop 1976; Bothe et al. 1977; Burris and Peterson 1976).

Concurrent nitrogen fixation and hydrogen formation by blue-green algal nitrogenase necessitates the sharing of both energy (adenosine triphosphate) and reductant reserves. Competition for these resources implies that neither process will function at maximal rates when both substrates are available (Jones and Bishop 1976). One third of the energy and reductant used by nitrogenase in vivo may be lost in hydrogen production (Anderson and Shanmugam 1977). The relevance of hydrogen production to rate comparisons would be negligible if substrate competition with hydrogen occurred during acetylene reduction as well. However, it has been determined that acetylene, supplied in concentrations sufficient for nitrogen fixation assays, inhibits nitrogenase-mediated hydrogen formation, allowing reservation of ATP and reductant for reduction of acetylene (Schubert and Evans 1976; Bothe et al. 1977). Therefore, acetylene reduction rates would not reflect a balance between hydrogen formation and ethylene production. Consequently a maximal reduction rate would be anticipated when this technique was used. Assuming that hydrogen production is characteristic of the species for which the rate comparison was undertaken in this study, responsibility for enhanced ratios might reasonably be attributed to this process. The efficiency of this hydrogen evolution is also influenced by the presence within a given algal speices of a cellular hydrogenase capable of uptake and oxidation of hydrogen produced by the nitrogenase (Anderson and Shanmugam 1977). Blue-green algal hydrogenases varied both in type and activity not only from organism to organism, but also from strain to strain (Bothe et al. 1977). Therefore, nitrogen-15 uptake rates would be reduced relative to acetylene reduction estimates by an amount dependent upon the extent of hydrogen formation and cellular hydrogenase activity.

Though use of the acetylene reduction technique for nitrogen fixation rate estimations is a convenient and economical method for analysis of a wide range of nitrogen-fixing organisms, it has been demonstrated in this study and others (Peterson and Burris 1976; Bergersen 1970; Lean et al. 1976) that assumption of a fixed acetylene to nitrogen conversion factor, which has been common practice, is unjustifiable.

An acetylene to nitrogen ratio of approximately 7.5 moles of acetylene reduced per mole of nitrogen fixed characterized the relationship of acetylene reduction and nitrogen-15 fixation for the freshwater blue-green algal populations in the ELA lakes examined. Use of this ratio rather than the stoichiometric value will reduce acetylene reduction-derived estimates of the contribution of nitrogen fixation to lake nitrogen budgets by a factor of about 2.5. Nitrogen fixation contributions estimated in 1975 (Flett et al. 1980) will decrease from 19% to 8% for Lake 226 NE and from 14 to 6% for Lake 227, a significant correction.

Though the similarity among lake acetylene to nitrogen ratios determined here suggests the possibility of ratio extrapolation among lake systems, it should be remembered that the ELA lakes examined exhibit many common characteristics, increasing the probability of a common response to both nitrogen fixation estimations. As discussed earlier, independent nitrogen-15 uptake calibration of the acetylene reduction method for a particular system is desirable due to the variable nature of the ratio and the differences in analytical procedures employed by individual investigators.

While the acetylene reduction technique as originally developed, with its assumption of a rigid stoichiometric conversion factor, is imprecise, discontinuation of the use of the technique is not suggested. Use of the acetylene reduction method to provide rapid sensitive nitrogen fixation estimates will still be an advantage,

especially in field studies. However, if an accurate estimate of nitrogen fixation is required, use of the isotope uptake method is preferable. If, for reasons of expediency, the acetylene reduction method is adopted as a routine nitrogen fixation assay procedure, the importance of precalibration of this method in each system with the nitrogen-15 uptake method is stressed. Without this preliminary method calibration, acetylene reduction rates should be reported as μg ethylene produced $\cdot L^{-1} \cdot h^{-1}$, rather than μg nitrogen fixed $\cdot L^{-1} \cdot h^{-1}$, following introduction of the untested theoretical ratio.

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Nitrogen Fixation in Canadian Precambrian Shield Lakes

ROBERT J. FLETT[1]

Microbiology Department, University of Manitoba, Winnipeg, Man. (at the Freshwater Institute)

DAVID W. SCHINDLER AND ROBERT D. HAMILTON[2]

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

AND NORMAN E. R. CAMPBELL

Microbiology Department, University of Manitoba, Winnipeg, Man.

FLETT, R. J., D. W. SCHINDLER, R. D. HAMILTON, AND N. E. R. CAMPBELL. 1980. Nitrogen fixation in Canadian Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 37: 494–505.

Acetylene reduction assays in the water columns of several artificially eutrophied lakes in the Experimental Lakes Area revealed that nitrogen fixation by planktonic blue-green algae could supply a significant portion of the nitrogen income to these lakes. In situ $^{15}N_2$ uptake experiments in one lake indicated that nitrogen fixation was proceeding at a slow rate in the low oxygen region of the thermocline, probably via methane oxidizing bacteria. Other $^{15}N_2$ uptake experiments in the littoral sediments of an oligotrophic lake failed to detect nitrogen fixation. Generally, algal nitrogen fixation occurred in Shield lakes that were subject to total N:total P loading ratios (wt/wt) of less than approximately 10. This suggests that in these lakes phosphorus removal from loading is the best technique for eutrophication abatement and that nitrogen removal, when applied by itself, may be detrimental because it could encourage bloom formation of nitrogen fixing blue-green algae.

Key words: nitrogen fixation, eutrophication, blue-green algae, acetylene reduction, nitrogen-15

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Des essais par réduction d'acétylène dans la colonne d'eau de plusieurs lacs artificiellement eutrophiés de la région des lacs expérimentaux indiquent que la fixation d'azote par les algues bleu-vert planctoniques pourrait fournir une portion appréciable des apports d'azote dans ces lacs. Des expériences d'absorption de $^{15}N_2$ in situ dans un lac indiquent que la fixation de l'azote se poursuit à un rythme lent dans la région pauvre en oxygène de la thermocline, probablement par le biais de bactéries oxydant le méthane. D'autres expériences d'absorption de $^{15}N_2$ dans les sédiments littoraux d'un lac oligotrophe ne purent déceler de fixation d'azote. En général, la fixation de l'azote par les algues se produit dans les lacs du Bouclier sujets à des rapports de charge N total:P total (poids/poids) inférieurs à environ 10. Ceci porte à croire que dans ces lacs, l'enlèvement du phosphore de la charge est la meilleure façon de ralentir l'eutrophisation et que l'enlèvement de l'azote, par lui-même, peut être préjudiciable car il pourrait encourager la formation de fleurs d'algues bleu-vert fixatrice d'azote.

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IN 1969, the International Joint Commission (I.J.C. 1969) adopted a eutrophication control policy stating, in part, that phosphorus rather than nitrogen should be removed from the influents to the Laurentian Great Lakes because phosphorus was the less expensive and less difficult of the two nutrients to remove. In addition, it was stated that nitrogen removal might be fruitless because biological atmospheric nitrogen fixation, an essentially uncontrollable process, could be supplying significant amounts of this nutrient to the lakes. More recently, atmospheric carbon dioxide has been shown to readily dissolve into lakes and subsequently be fixed by phytoplankton (Emerson et al. 1973; Schindler et al. 1973; Schindler and Fee 1973) and this indicates that the removal of carbon from influents will not cause that nutrient to become an algal growth limiting factor.

The magnitude of biological fixation of dissolved atmospheric nitrogen in waters of the Laurentian Great Lakes has not been clearly demonstrated. A brief study by Howard et al. (1970) showed algal nitrogen fixation occurred in Lake Erie but no estimate of its quantitative

[1]Present address: Flett Research Ltd., 9-30 Baylor Ave., Winnipeg, Man. R3T 3K1.

[2]To whom reprint requests should be addressed.

[3]Natural external loading to the ELA lakes discussed in this paper is about 0.06 g P and 1.1 g $N \cdot m^{-2} \cdot a^{-1}$.

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importance was made. A later, more intensive study by Vanderhoef et al. (1974) showed that algal nitrogen fixation supplied a small part of the nitrogen income to Green Bay, Wisconsin. There was no strong evidence to support the I.J.C. position that nitrogen removal from the influents to these lakes was a poor procedure for eutrophication abatement. A primary purpose of this study was to determine, for waters chemically and biologically similar to the Great Lakes, whether nitrogen fixation could supply this nutrient in sufficient quantities to possibly prevent nitrogen limitation of algal growth if other sources of assimilable nitrogen were removed from influents.

To answer this question, a variety of lakes, experiencing different nutrient loadings, were examined at the Experimental Lakes Area (ELA) of northwestern Ontario. The ELA is an ideal region for limnological research because the lakes are isolated from cultural effects of man and the lake basins are bedrock controlled, a situation that allows for the construction of accurate nutrient budgets (Johnson and Vallentyne 1971). These ELA lakes are of more than local interest because they and literally thousands of others, among them the Laurentian Great Lakes, share a common trait in that the Precambrian Shield drainage waters enter them all. This similarity allows some careful extrapolation of conclusions reached in the small experimental lakes to the larger and economically important Great Lakes and to innumerable other Shield lakes across Canada.

An effort was made to determine the nitrogen fixation rates of the plankton, and furthermore, to learn whether bacteria or blue-green algae were the major fixing agents. Other efforts involved examination of the sediments to determine if they were possible sites of nitrogen fixation. Both acetylene and $^{15}N_2$ techniques were employed to measure nitrogen fixation, sometimes concurrently, it being recognized that neither technique was completely adequate in all situations.

The Lakes Studied

Three lakes, 227, 302 South, and 261 were regularly monitored at the ELA for planktonic N_2 fixation beginning in May 1973 and similar studies were begun on Lake 226 Northeast in late August of that year. The study was carried out over 3 ice-free seasons until fall 1975.

Lake 226 is a well stratified double basin lake (Table 1) and was classified as oligotrophic prior to 1973 when a fertilization program was initiated. In the spring of 1973 the two lake basins were separated by positioning a plastic Sea Curtain (Kepner Plastics) across the lake at the narrow neck. This effectively created two separate lakes. The Southwest Basin was continuously fertilized over a 21-wk period each year over the open water season with 5.89 g C·m^{-2}·a^{-1} as sucrose and 3.08 g N·m^{-2}·a^{-1} as $NaNO_3$. The northeast basin was similarly fertilized except that in addition to the C and N, a further 0.58 g P·m^{-2}·a^{-1} was added as phosphoric acid.[3] The northeast basin rapidly responded to the nutrient additions and algal biomass increased above that in the other basin (Schindler 1974). Toward the end of August 1973 a thick epilimnetic bloom of *Anabaena spiroides* appeared and in subsequent years nitrogen fixing blue-greens were major components of the plankton under the continued fertilization (Schindler 1977).

Table 1. Morphometric data for the lakes studied in the Experimental Lakes Area.

Lake	Area (ha)	Mean depth (metres)	Max. depth (metres)	Nutrients added artificially
226 NE	8.32	5.7	14.7	CNP
226 SW	7.77	6.3	11.6	CN
227	5.00	4.4	10	NP
239	56.1	10.5	30	none
261	5.57	2.9	9	P
302 S	10.9	5.1	10	none
302 N	12.8	5.7	13	CNP

Lake 227 is a small well stratified lake (Table 1) that, prior to 1969, in its natural state was oligotrophic (Schindler et al. 1971) with averaged analyses from the euphotic zone showing total dissolved nitrogen = 160 μg·L^{-1}, total dissolved phosphorus = 7 μg·L^{-1}, and chlorophyll *a* = 2–5 μg·L^{-1} (Armstrong and Schindler 1971). Chrysophyceae were the dominant phytoplankton. In the period from 1969 to 1974 a program of fertilization was in effect (Schindler et al. 1971, 1973) wherein additions of 0.48 g P·m^{-2}·a^{-1} (as H_3PO_4) and 6.29 g N·m^{-2}·a^{-1} (as $NaNO_3$) were added in 20–21 weekly increments from mid-May to mid-October. The lake responded immediately to the increased nutrient loading and exhibited a higher algal standing crop, an elevated chlorophyll *a* concentration and a shift in population to chlorophytes. Cyanophytes were abundant but were non-nitrogen fixing species such as *Oscillatoria redekei* (Schindler et al. 1973).

In 1975 an attempt was made to induce a bloom of nitrogen fixing algae by reducing the nitrogen:phosphorus loading ratio to a level similar to that in Lake 226 Northeast. This new fertilization program (0.46 g P·m^{-2}·a^{-1} and 2.25 g N·m^{-2}·a^{-1}) did stimulate a large population of nitrogen fixing *Aphanizomenon gracile* (Schindler 1977).

Lake 302 is a well stratified double basin lake (Table 1) with the basins separated by a plastic Sea Curtain in a manner similar to Lake 226. During the period 1973–75 the South Basin received no fertilizer while the North Basin received total additions to the hypolimnion of 3.73 g C·m^{-2}·a^{-1} (as sucrose), 0.54 g P·m^{-2}·a^{-1} (as H_3PO_4) and 2.79 g N·m^{-2}·a^{-1} (as NH_4Cl) (Schindler 1975) in weekly increments over the summer. Both basins have remained oligotrophic and at no time were significant numbers of nitrogen

fixing algae observed in biweekly samples (D. W. Schindler and T. Ruszczynski personal communication).

Lake 261 is small (Table 1) and well stratified and was fertilized weekly over the summer with H_3PO_4 at a rate of 0.246 g $P \cdot m^{-2} \cdot a^{-1}$ from 1973 onward. The lake was classified as oligotrophic prior to fertilization and mesotrophic afterward. No significant populations of nitrogen fixing blue-green algae were observed in monthly samples (Schindler 1977). Lake 239 (Table 1) is an undisturbed, naturally oligotrophic lake that has been carefully monitored for nutrient input and export (Schindler et al. 1976). The littoral zone of this lake was chosen for the in situ measurement of $^{15}N_2$ fixation in the sediments.

Materials and Methods

Acetylene Reduction Assays for Nitrogen Fixation

Acetylene reduction in the water column — In situ planktonic nitrogen fixation was measured weekly at a central station in each lake by acetylene reduction, using a syringe technique described by Flett et al. (1976). In 1973 and 1974 samples were obtained by a 3-L Van Dorn bottle. In 1975 a portable peristaltic pump was used instead because it obtained samples from more precisely defined depths. Metre length sections of 0.95 cm OD Tygon tubing (Norton Plastics) were connected together by plastic unions until the hose was the correct length for sampling the desired depth. This assured that hose length was as short as possible, an important factor if degassing of methane, nitrogen, oxygen, etc. from the water because of pressure and temperature changes, is to be avoided (Rudd and Hamilton 1975). The sampling hose was suspended from a plastic float so that vertical movements of the boat were not transmitted to the hose. The pump fed into the bottom of an opaque Plexiglas vessel of 500 mL volume which overflowed from the open top. This opaque vessel partially protected the water from bright surface light yet permitted easy subsampling of the water, by syringe, through the top. Sufficient pumping time (30 s) at each new depth was allowed for the vessel to be theoretically filled 3 times. This eliminated "memory" of previous samples. Gas exchange with the atmosphere was judged to be minimal because Winkler oxygen determinations showed anoxic bottom water samples could be taken in the syringes.

The syringes, in duplicate, were incubated in situ at the depths of collection (0, 1, 2, 3, 4, 5, 7, 9 m) for 2–4 h, generally between 10:00 and 14:00. The incubations were concluded by placing the syringes into a light tight box in which they were transported to the laboratory where processing was completed within an hour. Minimal fixation was noted to occur during transport.

Computer modeling — As measurements of acetylene reduction were only done weekly, considerable interpolation between measurements was necessary. Early experimentation indicated that nitrogen fixers in these lakes were photosynthetic and therefore were strongly under the influence of changing incident solar radiation. For this reason it was considered important to relate acetylene reduction directly to incident light to account for the many variations in light due to cloud cover and day length (Schindler and Fee 1975). Incident light was measured at the ELA meteorological station for the duration of this study. Light extinction coefficients, based upon biweekly measurements, were available for each lake being studied (Shearer 1976; Shearer and DeClercq 1976). These allowed calculation of the light available for fixation at any depth, as a function of incident solar radiation. Relationships between light intensity and rate of acetylene reduction were obtained for samples from Lake 226 Northeast in 1974 and 1975 using the light gradient incubator of Fee (1973).

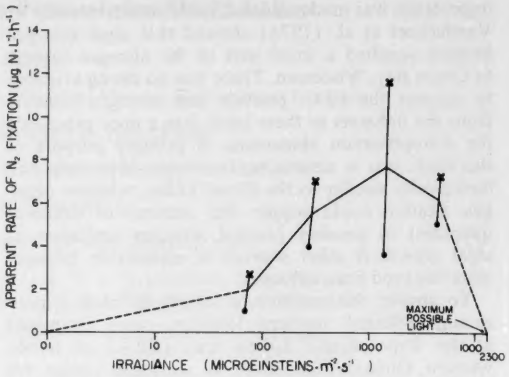

FIG. 1. Average (for 2 dates) nitrogen fixation rates as functions of light intensity in 1.0 m depth samples, Lake 226 NE on August 10 (×) and 21 (●), 1974. The incubations were done in the Fee incubator for 2 h, the samples having been taken about 10:00. Dashed lines are explained in the text.

Figures 1 and 2 are plots of average nitrogen fixation rates as functions of light intensities. A ratio of one nitrogen molecule reduced per three ethylene molecules produced was assumed. The dashed lines were arbitrarily chosen to complete the fixation:light relationship for low and high light intensities, where actual measurements could not be made. For 1974, the decision to make fixation rates equal zero at 0.1 $\mu E \cdot m^{-2} \cdot s^{-1}$ or lower light intensity was based upon in situ results which showed fixation to be just detectable (0.025 $\mu g \cdot L^{-1} \cdot h^{-1}$) at light levels of 0.1 $\mu E \cdot m^{-2} \cdot s^{-1}$ on August 17, 1974, a date intermediate to the two incubator studies. The dashed line at the high light intensities was plotted so that fixation would equal zero if the fixing organisms experienced maximum possible irradiance from the sun (2300 $\mu E \cdot m^{-2} \cdot s^{-1}$) at the very surface of the lake. This presumed that the organisms would exhibit 100% light inhibition under such circumstances, a rather unlikely presumption. Both of these arbitrarily positioned dashed lines were assumed to underestimate nitrogen fixation rates. For 1975 the assumptions were the same except that fixation was extrapolated through the two lowest irradiance levels to equal zero when the light was 0.38 $\mu E \cdot m^{-2} \cdot s^{-1}$. It was assumed that the relationship between incident light and nitrogen fixation, as seen in the curves of Fig. 1 and 2, was valid over the entire summer period that fixation was measured.

The program functioned as follows: For each day in which in situ acetylene reduction results were positive, predictions of nitrogen fixation were obtained from the model

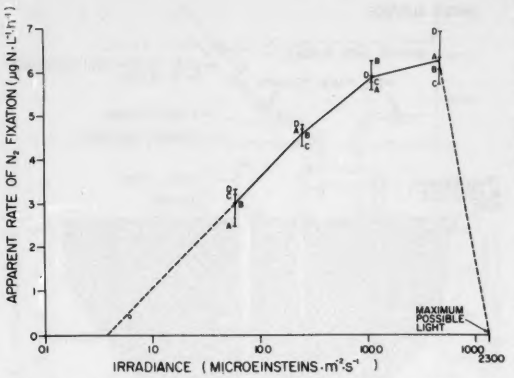

FIG. 2. Average nitrogen fixation rates as functions of light intensity, for integrated Lake 226 NE samples (0–5 m depth) on Sept. 12, 1975. Samples were taken at 06:00, 10:00, 14:00, and 17:00 and incubated for 2 h in the Fee incubator. The results of all the different sampling periods were averaged to give the plot illustrated here. Dashed lines are explained in the text. A = 06:00, B = 10:00, C = 14:00, and D = 17:00.

for the same time periods over which the in situ assay was performed. The predicted fixations at 0.2, 1.2, 2.2, 3.2, 4.2, and 5.2 m depths were compared with the observed fixations at the same depths and a factor was calculated for each depth that expressed the observed value as a fraction of the predicted value. Next, the model was asked to predict the fixation rates at discrete depths over the whole lighted day, at 0.2-m intervals. The predicted value at each depth was multiplied by the previously obtained factor from the same or closest depth. For example, the factor obtained from the in situ depth of 1.2 m would be used to multiply the model predictions at 0.8, 1.0, 1.4 and 1.6 m depths. Similarly, the factor obtained from the 2.2 m depth in situ incubation was used to multiply the model predictions from 1.8, 2.0, 2.2, 2.4, and 2.6 m depths, etc.

The rates of adjacent 0.2 m depths were then averaged and this value was used to multiply the total lake volume enclosed between the two depths. This was performed for depths from 0.0 to 5.0 m, the only depths for which significant activity was noted. Twenty-five depths were summed and the resultant value represented the total day's fixation of nitrogen into the lake.

The model was then asked to calculate fixation for days other than those when in situ fixation data were available. The assumption was made that all factors except incident radiation remained constant with time and therefore the only new input required for the program to run was the light data for each day examined. In situ fixation measurements were normally done once weekly; the factors derived from these measurements were applied to the three days preceding and the three days following the in situ measurements.

The program produced daily estimated totals of fixation and the sum of these totals represented the estimated yearly total input of fixed atmospheric nitrogen to the lake.

Acetylene reduction in sediment slurries — In Lake 302 South (Oct. 8, 1973) and Lake 227 (several occasions in July 1974) sediment slurries were obtained by allowing a 3-L Van Dorn sampler to partially penetrate the soft bottom sediments of the hypolimnia before being triggered (10.5 m in Lake 302 S, 8.0 m in Lake 227). The slurries were assayed for nitrogen fixation by the acetylene reduction syringe technique in the same manner used for water samples. The samples were incubated for about 4 h in situ just above the lake bottoms. The hypolimnetic waters of both lakes were anoxic.

$^{15}N_2$ Assays for Nitrogen Fixation

Occasional direct measurements of $^{15}N_2$ uptake were made using an emission spectrometric method that was substantially different from those of previous workers (Dugdale et al. 1959). A Statron NOI-5 N-15 analyser was employed for the isotopic measurements because it required very small amounts of sample, a prime consideration when concentration of samples by filtration or centrifugation was thought undesirable. Using a preparation unit devised in this study, samples (on glass fibre filters) containing as little as 5 μg of nitrogen could be analyzed for ^{15}N content with a precision of ± 0.02 atom % (95% C.I. at natural abundance) (Flett 1977). The total quantity of nitrogen in the sample was concurrently determined by the preparation unit thereby permitting easy measurement of absolute rates of nitrogen fixation.

Calibration of the acetylene reduction technique — It was thought necessary to determine if the commonly accepted 3 to 1 ratio of C_2H_2:N_2 reduction rates was accurate in this particular study. For this reason, several direct comparisons of the two methods were made. The comparisons were performed on subsamples of larger samples taken by peristaltic pump from Lake 227 and 226 NE on various dates. The acetylene reductions were always done in syringes with 30 mL of water as previously described by Flett et al. (1976). For the $^{15}N_2$ uptake, if the particulate nitrogen content of the sample was $<200\ \mu g\ N \cdot L^{-1}$, 120-mL serum bottles were filled with sample, capped with serum stoppers (no air bubbles) and then 4.0 mL of $^{15}N_2$ (57–95 atom %) were added (Fig. 3A, B). If the particulate nitrogen content was $>200\ \mu g\ N \cdot L^{-1}$, 30-mL samples were sucked into 50-mL glass syringes, 1.0 mL of $^{15}N_2$ (95 atom %) was added (Fig. 3A, C) and the syringe next sealed with a plugged hypodermic needle. Vigorous agitation of at least 5 min was required to equilibrate the $^{15}N_2$ with the previously dissolved atmospheric nitrogen, in both bottles and syringes.

The samples were next fastened to a clear Plexiglas rod horizontally suspended between two floats at approximately 0.2 m depth for periods shorter than 5 h in situ. Quadruplicate $^{15}N_2$ and duplicate acetylene reduction samples were incubated together on the same rod so that comparisons could be made between rates obtained from each method. At the end of the incubation, all the samples were immediately removed from the light; the $^{15}N_2$ samples were filtered within one hour through precombusted (550°C — 18 h) Reeve-Angel 984 H glass fibre filters. The filters were placed in sealed 5 cm Petri dishes, quickly frozen and stored at −40°C until analysis. The acetylene reduction samples were also processed within 1 h of their removal from the light. To determine if the C_2H_2:N_2 ratio of 3:1 held for various light intensities, on one occasion duplicate $^{15}N_2$ and acetylene reduction assays were per-

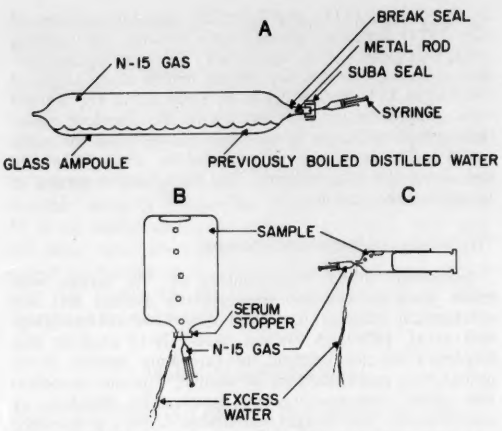

FIG. 3. A diagram showing how $^{15}N_2$ gas was: (A) obtained from the gas ampoule, (B) added to the sample in the serum bottle, (C) added to the sample in the glass syringe.

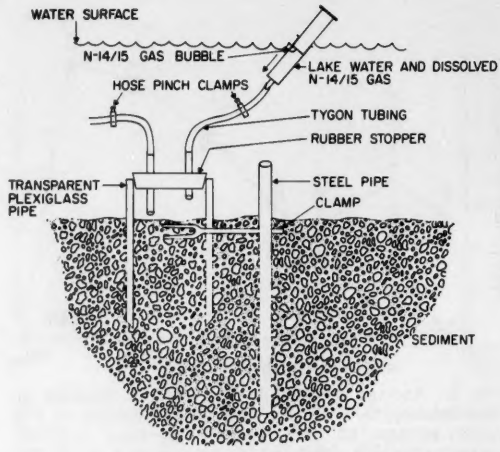

FIG. 4. The apparatus used to isolate sediment cores and then enrich them with $^{15}N_2$ gas.

formed on subsamples (all in syringes) at four different light levels in a Fee (1973) incubator.

$^{15}N_2$ fixation by CH_4 oxidizers in the water column — Methane oxidizing bacteria are active in some lakes of the ELA at depths where the oxygen concentrations are between 1.0 and 0.1 mg $O_2 \cdot L^{-1}$ (Rudd et al. 1974). Some methane oxidizing bactria fix nitrogen (Davis et al. 1964) and therefore it was decided to investigate the possibility that the methane oxidizing bacteria in these lakes were also nitrogen fixers. The $^{15}N_2$ technique was chosen to assay for nitrogen fixation because we suspected that the acetylene reduction technique was not applicable to the methane oxidizers (Flett et al. 1975). Such a view has recently been reinforced by de Bont and Mulder (1976). The $^{15}N_2$ method used here was the same as that employed for calibration of the acetylene reduction assay.

An attempt was made to demonstrate in situ $^{15}N_2$ fixation by methane oxidizers in Lake 227 (Sept. 26, 1974) where CH_4 oxidation had been noted to occur. A sample of 120 mL volume, taken from 4.75 m depth and containing 1.02 mg $O_2 \cdot L^{-1}$ was equilibrated with 5 mL of 57 atom % $^{15}N_2$ gas, incubated in situ for 6 h and then filtered. The filter was then analyzed for N-15 content by emission spectrometry. A second attempt was made to show this type of nitrogen fixation, this time in Lake 302 N because the hypolimnion of this lake basin was receiving fertilizer additions and was anoxic during summer stratification and therefore likely to support a population of CH_4 oxidizers at the oxic/anoxic interface. On July 31, 1975, samples of 120 mL volume were taken at different depths of the low oxygen zone; each sample was equilibrated with 4 mL of 97 atom % $^{15}N_2$ and then incubated in situ for 18 h before filtering and analysis for N-15.

$^{15}N_2$ fixation in sediment cores — Littoral sediments at 0.3 m depth, rich in decaying plant matter, were examined for $^{15}N_2$ fixation in the southeast subbasin of Lake 239. A 1 × 2 m working platform was constructed just above the water surface. A steel pipe of 2 cm diameter and 1 m length was driven about 80 cm into the sediment beside the platform. Onto the pipe was fitted a clamp capable of holding a vertical pipe of 7.0 cm diameter. A 20 cm length of 7 cm diameter Plexiglas pipe, with a beveled leading edge, was forced very gently into the sediment, through the open arms of the clamp (Fig. 4). Once the leading edge of the pipe was to a sediment depth of 15 cm, the clamp was closed so that the pipe was firmly held in position. The pipe was allowed to stand for at least 2 wk before any further manipulations were performed, in an attempt to allow the sediments to resume their undisturbed state. After this waiting period the pipe was gently capped with a large stopper that had two glass tubes through it (Fig. 4). About 20 cm of Tygon tubing had previously been attached to each of the glass tubes, and on each piece of Tygon tubing was an open hose pinch clamp.

Into a 50-cm^3 glass syringe was drawn 50 mL of water from the lake immediately adjacent the pipe. To this syringe was added 2.0 mL of 95 atom % $^{15}N_2$ gas from a second 5-cm^3 syringe (Fig. 3C). The syringe was sealed with a needle forced into a stopper and shaken vigorously for 5 min by hand. The same process was repeated for a second 50-cm^3 syringe. The needle was next removed from one of the 50-cm^3 syringes and the $^{15}N_2$ containing lake water was gently forced into the stoppered pipe through one of the Tygon tubes, as was the equilibrated $^{15}N_2/^{14}N_2$ gas bubble, an equal volume of water being displaced from the other Tygon tube. The bubble contained, in addition to the nitrogen, some CH_4, CO_2, and O_2 removed from the water during equilibration; by reintroducing the gases they were again available to the organisms in the tube should they be needed. This was repeated for the second syringe so that the desired 100 mL of $^{15}N_2$ containing water was added and then both hose pinch clamps were closed. The aqueous volume of the core was about 100 mL.

After a 2-wk incubation period, the hose pinch clamps were opened and the stopper gently removed from the Plexiglas pipe. A tiny piston corer, constructed from a 10-cm^3 syringe, was used to obtain a 1.5 cm diameter by 5 cm

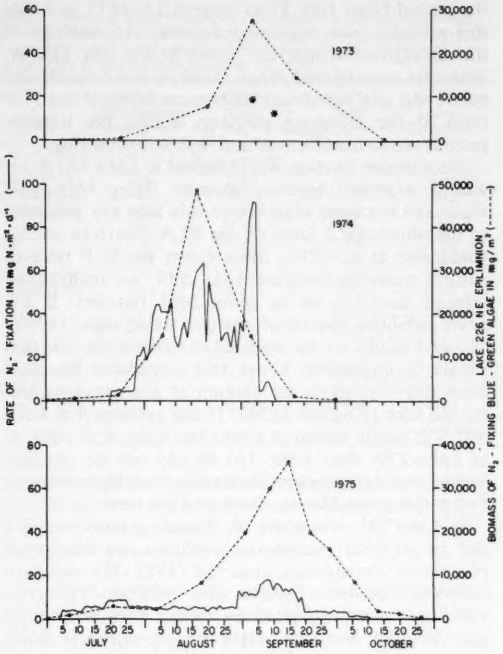

FIG. 5. Wet weight biomass of the nitrogen fixing blue-green algae (dashed lines) and nitrogen fixation (acetylene reduction) rates (asterisks denote single data points, solid lines denote modelling) over 3 yr in Lake 226 NE. (Top panel — 1973, Data for Sept. 4 and 11 only; Middle panel — 1974, Bottom panel — 1975.)

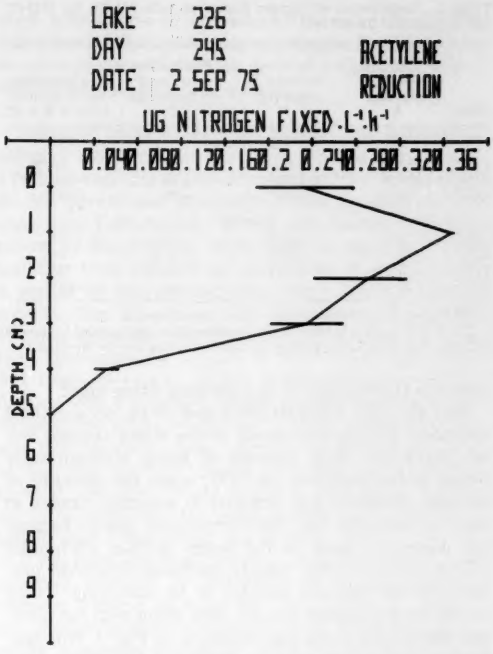

FIG. 6. A typical in situ nitrogen fixation depth profile for Lake 226 NE. The acetylene reduction technique was used and a C_2H_4:N_2 ratio of 3:1 was assumed. Bars join duplicates.

long core. Cores appeared to retain their integrity even after being transported back to the laboratory where they were immediately frozen by placing them vertically upright in a −40°C freezer for 2 h.

Once frozen, the core was extruded from the syringe in 0.3 cm increments and sections were carefully cut off by a clean jeweler's saw and deposited onto prefired (550°C for 18 h) Reeve-Angel glass fibre filters of 2.5 cm diameter. The filters were then held by clean forceps in front of an air stream from a Heat Gun (Master Appliance Corporation) until the samples were dry (approximately 1 min). The samples were stored in 5 cm diameter Millipore disposable Petri dishes at room temperature until analysis.

For nitrogen isotope analysis, several cubic millimetres of the dry sediment were placed on another prefired Reeve-Angel 984 h glass fibre filter and analyzed for ^{15}N content by emission spectrometry (Flett 1977).

Results and Discussion

Acetylene Reduction Assays

Blue-green algae — Significant rates of acetylene reduction were detected only in the presence of nitrogen fixing algae and the rates showed a strong dependence upon light intensity. The asterisks in Fig. 5 show the results of the acetylene reduction depth profiles obtained on Sept. 4 and 11, 1973 in Lake 226 NE. The data were insufficient to permit using the computer model but other experiments had indicated that, in this lake, the total nitrogen fixed in a day could be estimated by assuming the 10:00 to 14:00 rate to be valid for 10 h/d. Using this relationship and taking into account the volume and area of the lake, rates of fixation during the two days were calculated (assuming C_2H_2:N_2 ratios of 3:1). The biomass of the *Anabaena spiroides* bloom, induced by the fertilizer, is shown also. In 1974, the weekly data were sufficient to permit modeling of fixation (Fig. 5). The dominant blue-green alga was *Anabaena solitaria* f. *planktonica*. In 1975 a bloom of the same species again appeared and it was assayed weekly as in 1974 and modeled (Fig. 5). The algal biomass and species identification are due to Findlay (1978). Figure 6 shows a typical acetylene reduction depth profile.

In Lake 226 NE, nitrogen fixation obviously had some importance, ranging from 7 to 38% of total inputs. Table 2 shows the estimated masses of nitrogen fixed in the lake for the years of 1973–75. The amount of fixation in 1974 and 1975 was generally propor-

TABLE 2. Contribution of nitrogen fixation to Lake 226 NE for 1973–75 and to Lake 227 for 1972–75.

Year	N_2 fixation input (kg) A	Fertilizer N input (kg) B	Precipitation and runoff N input (kg) C	% contributed by N_2 fixation A/(A + B + C)
		Lake 226 NE		
1973	17.8[a]	149.7	72.5	7.5%
1974	139.6	149.7	76.2	38.2%
1975[b]	51.4	149.7	~70	~19%
		Lake 227		
1972	0	314.2	61.9	0%
1973	0	314.2	47.6	0%
1974	0	314.2	66.8	0%
1975[b]	26	105	~60	~14%

[a]Minimum estimate based upon 2 d of measurements.
[b]1975 total loading is based upon the assumption that natural loading in 1975 was the same as the average of natural loading for the previous 3 yr.

tional to the biomass of the nitrogen fixing algae.

In Lake 227, through 1973 and 1974, no acetylene reduction activity was noted in the water column and no blue-green algae capable of fixing nitrogen were found in the plankton. In 1975, when the quantity of nitrogen fertilizer was reduced to amounts similar to that in Lake 226 NE, *Aphanizomenon gracile* became the dominant algae in the water column (Schindler 1977). Concurrently, weekly acetylene reduction profiles showed nitrogen fixation to be occurring. These results were modeled and are seen along with the nitrogen fixing blue-green algal biomass, in Fig. 7. Nitrogen fixation was calculated to contribute 14% of the total input to the lake in 1975 (Table 2).

The bimodal shape of the Lake 227 fixation curve (Fig. 7) is not easily explained. It is possible that NH_3 derived from decaying organic matter was being returned to the water column from the sediments during the period from July 25 to August 13, 1975 and that this nitrogen was repressing fixation. An analysis of the allochthonous nutrient inputs to the lake (D. W. Schindler unpublished data) (total N and total P) did not reveal any significant differences between the N:P ratio of the incoming nutrients during the summer periods when fixation was and was not occurring.

No nitrogen fixation was observed in Lake 302 S nor was it expected because nitrogen fixing blue-green algae had not been observed in this lake nor generally in the oligotrophic lakes of the ELA. Previous studies (Schindler et al. 1976) have shown the N:P ratio of natural nutrients entering Lake 239, an undisturbed lake in the ELA, to be about 20:1 (wt/wt). It has never exhibited blooms of nitrogen fixing algae (1969–76) and would not be expected to because the nitrogen supply is appreciably above that considered for good algal nutrition, given the amount of phosphorus entering the lake (Redfield 1934). If one assumes that Lake 302 S is being loaded at about the same N:P ratio as in Lake 239, then Lake 302 should not be nitrogen limited and furthermore, it should not exhibit nitrogen fixing blue-green blooms. Such was the case.

In Lake 261, where the N:P loading ratio was 5:1 due to artificial phosphorus additions, no blue-green planktonic blooms were observed (1973–75) nor were acetylene reduction assays ever positive. This was puzzling because the plankton should have been nitrogen deficient and one might expect nitrogen fixing algae to prosper in such an environment. In 1975, however, it was found that a periphyton community of *Anabaena* sp. was actively fixing nitrogen on the lake bottom (M. Turner, R. Flett, and H. Holloway unpublished data). It is tempting to suggest that these nitrogen fixers were a result of the low N:P loading

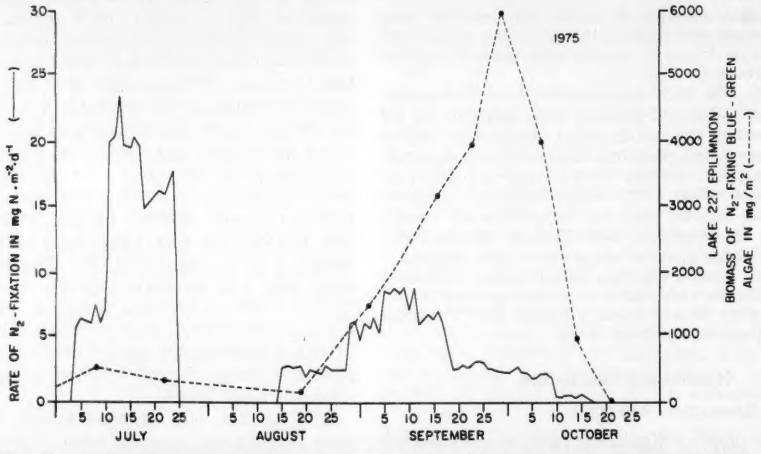

FIG. 7. A plot of nitrogen fixation rates (solid lines) and nitrogen fixing blue-green algal biomass (dashed lines) as a function of time in Lake 227, 1975. The fixation rates were predicted using a computer model described in the text.

TABLE 3. Rates of nitrogen fixation as determined by the acetylene reduction and isotopic methods when applied to subsamples of the same water. Data marked with * are excluded from the average because it is believed that the activity was too low to be measured accurately.

Date	Incubator light intensity $\mu Es \cdot m^{-2} \cdot s^{-1}$	Rate by C_2H_4 $\mu gN \cdot L^{-1} \cdot h^{-1}$	Rate by N-15 $\mu g\,N \cdot L^{-1} \cdot h^{-1}$	Ratio of rates $C_2H_4/N\text{-}15$
		Lake 226 NE		
Sept. 4, 1974	—	2.02	0.51	3.96
Sept. 4, 1975	—	0.261	0.123	2.12
Sept. 12, 1975	462	0.574	0.193	2.97
	102	0.578	0.165	3.50
	25	0.433	0.143	3.03
	6	0.322	0.036	8.94*
Sept. 18, 1975	—	0.247	0.108	2.29
Sept. 24, 1975	—	0.116	0.047	2.47
			Average =	2.90
			Excluding*	
		Lake 227		
July 21, 1975	—	0.99	1.40	0.71
Sept. 18, 1975	—	0.24	0.088	2.73
Sept. 25, 1975	—	0.095	0.046	2.05
			Average =	1.83

ratio although such a conclusion is impossible to substantiate because the periphyton were not examined for nitrogen fixation activity in years prior to the phosphorus additions.

Sediment slurries — No acetylene reduction activity occurred in either of the anaerobic sediments of Lake 302 S or Lake 227. The negative results observed in both lakes must be viewed with caution because the performance of the acetylene reduction assay in anaerobic (Brouzes and Knowles 1971; Oremland and Taylor 1975) and nearly anaerobic environments (Flett et al. 1975; de Bont and Mulder 1976) is questionable. During sampling in Lake 302 S the NH_3–N concentration was 2400 $\mu g \cdot L^{-1}$ while in lake 227 the concentration ranged from 2400 to 2800 $\mu g\,N \cdot L^{-1}$ at sampling depths. Although great confidence cannot be placed in the fixation measurements, it appears doubtful that fixation was occurring in those environments where NH_3 was already present in abundance.

$^{15}N_2$ Assays for Nitrogen Fixation

Comparison of acetylene reduction to $^{15}N_2$ uptake — The results of the comparisons between the two nitrogen fixation assays for planktonic algae are seen in Table 3. It is obvious that the acetylene reduction technique, using the ratio of 3 C_2H_4 produced:1 N_2 fixed, usually gave much higher estimates of fixation.

As the $^{15}N_2$ uptake method used here measured the increased content of the particulate material only, the discrepancy between the methods could be explained by algal excretion of already fixed N–15 or perhaps by cell breakage on the filters. Alternatively, the 3:1 ratio may be incorrect. A more complete treatment of this problem can be found in Graham et al. (1980).

Table 3 indicates that acetylene reduction is overestimating nitrogen fixation by a factor of more than two. If this were so, all the budget figures given in this work would have to be reduced by more than 50% and although the significance of nitrogen fixation would be substantially reduced, the general conclusions of this study would not be greatly altered.

Sediment cores — No $^{15}N_2$ fixation was detected in either of two cores examined from the littoral of Lake 239. However, the negative results for $^{15}N_2$ in the cores do not permit one to conclude that no fixation was occurring. Calculations, taking into account the sensitivity of the method, reveal that 2.8 mg $N \cdot m^{-2} \cdot d^{-1}$ could be fixed and still go undetected. If one assumes a period of fixation extending over the six warmer months, then 0.5 g $N \cdot m^{-2} \cdot a^{-1}$ could have been fixed but not measured. The loading rate of Lake 239 has been approximately 1.2 g $N \cdot m^{-2} \cdot a^{-1}$ (average 1972–74) (Schindler et al. 1976). If the sediment nitrogen fixation were actually occurring and at a rate just below detectability (0.5 g $N \cdot m^{-2} \cdot a^{-1}$), then sediment nitrogen fixation could have supplied significant portions of the total nitrogen input to the lake and still have escaped detection. More sensitive techniques are required to determine the importance of sediment nitrogen fixation (Macgregor and Keeney 1973).

It has been suggested that this assay may not give an accurate estimate of anaerobic or microaerophyllic nitrogen fixation because the $^{15}N_2$ might not diffuse far enough into the sediments to be taken up by the active organisms during the incubation period. Recent studies of sediment diffusion rates in another ELA lake (Hesslein 1976) indicate that the $^{15}N_2$ gas probably was present in substantial concentrations at depths of at least 2 cm by the end of the experiment. Methane bubbles were observed in these sediments and methane concentration data of Hesslein (1976) indicate that the sediments very probably are anaerobic at depths in excess of several millimetres. Therefore anaerobic $^{15}N_2$ fixation should have been detected if it were occurring at a measurable rate in the upper few centimetres of sediment. From this discussion it also follows that $^{15}N_2$ should have been present in high concentrations throughout the oxic/anoxic gradient (top few millimetres) thereby permitting accurate assay of anaerobic, microaerophyllic (CH_4 oxidizers) and aerobic $^{15}N_2$ fixation activity with rates greater than 2.8 mg $N \cdot m^{-2} \cdot d^{-1}$.

$^{15}N_2$ fixation by CH_4 oxidizers in the water column — In Lake 227 no $^{15}N_2$ fixation was detected in the low oxygen zone of the thermocline where microbial CH_4 oxidation was suspected to be proceeding. This was probably due to the oxygen concentration being slightly above the critical value of 1.0 mg $\cdot L^{-1}$. In Lake 302 N, however, slow rates of $^{15}N_2$ fixation were observed in the low oxygen zone (Table 4). The fixation was occurring in a hypolimnetic bloom of algae, concentrated in a narrow band around the 7.5 m depth, this concentration being easily noted in the N particulate data of Table 4. The dominant algal genera were, in order of importance, *Chlamydomonas*, *Cryptomonas*

TABLE 4. Nitrogen fixation rates, particulate nitrogen concentrations and oxygen concentrations as a function of depth in Lake 302 N, July 31, 1975.

Depth (m)	O_2 Concentration ($mg \cdot L^{-1}$)	Particulate N ($\mu g \cdot L^{-1}$)	N_2 Fixation ($\mu g\ N \cdot L^{-1} \cdot h^{-1}$)
7.2	0.2	295	0.011
7.4	0.1	371	0.019
7.6	0.1	475	0.019
8.0	0.0	340	0.006

and *Mallomonas* (E. J. Fee unpublished data). Because no nitrogen fixing species were noted, it was assumed that methane oxidizers were responsible for the fixation.

An attempt was made to estimate the possible magnitude of such a nitrogen input to the lake. As a first estimate, if one assumes average bacterial composition to be approximately 1 mol N:10 mol C (Vallentyne 1974), 50% incorporation of CH_4 (Rudd 1976) and 100% incorporation of N_2 fixed, then 20 mol of CH_4 would be oxidized for each mole of N_2 fixed. Therefore, given a CH_4 oxidation rate of $0.1\ \mu M \cdot L^{-1} \cdot h^{-1}$ (max. upper estimate — J. W. M. Rudd personal communication) occurring over 3 mo, then approximately $300\ \mu g\ N \cdot L^{-1}$ would be fixed. If one further assumed that the activity took place thrugh one metre of depth, then the contribution of nitrogen would have been about $0.13\ g\ N \cdot m^{-2}$. As a second estimate, if one took $0.015\ \mu g\ N \cdot L^{-1} \cdot h^{-1}$ as an average fixation rate (from Table 4), the contribution of nitrogen to this lake would have been $0.014\ g\ N \cdot m^{-2}$. The natural allochthonous input of nitrogen to the lake is about $0.8\ g\ N \cdot m^{-2} \cdot a^{-1}$ (Schindler unpublished data). There exists, therefore, only a slight possibility that this fixation might supply a significant fraction of the natural nitrogen input to the lake. If one includes the fertilizer nitrogen input in these calculations ($2.79\ g\ N \cdot m^{-2} \cdot a^{-1}$), the possible relative significance of this nitrogen fixation is further reduced. The quantity of nitrogen fixed may be significant to the methane oxidizers themselves but it is unlikely that it is very important to the nitrogen budget of the entire lake.

TABLE 5. Relationship of N:P loading by weight to the occurrence of blue-green fixers in Lake 227.

Year	Total N loading (kg)	Total P loading (kg)	N:P ratio loading	Presence of blue-green fixers
1972	376.1	26.1	14.4	−
1973	361.7	25.9	14.0	−
1974	381.0	26.9	14.2	−
1975[a]	~165	~26	~6.3	+

[a]1975 total loading is based upon assumption that natural loading in 1975 was the same as average natural loading over the previous 3 yr.

RELATIONSHIP OF NITROGEN FIXATION TO N:P: LOADING RATIOS

The data for Lake 227 in Table 5, relating the appearance of nitrogen fixing blue-green algae to the total N:total P loading ratio, suggest that lower N:P loading ratios may encourage development of blue-green algal blooms while higher N:P loading ratios may favour other algal types (Schindler 1977). To further investigate the possible relationship between the ratio of N:P loading and the presence of nitrogen fixing algae, data from a number of other lakes were examined to see if the relationship held for them also (Table 6).

TABLE 6. Average ratios of inorganic nitrogen concentrations: total dissolved phosphorus concentrations in both nutrient loadings and lake waters and the relationship of these ratios to the occurrence of blue-green nitrogen fixing algae for a number of lakes on various dates.

		Ratio (weight/weight) of			
Lake	Date	Tot. N:Tot. P in loading	Avg. DIN:TDP in summer epilimnion	Avg. Conc. DIN in summer epilimnion $\mu g\ N \cdot L^{-1}$	Presence of blue-green N_2 fixers
226 NE	1973	6.8	2.8	6.3	+
226 NE	1974	7.1	3.1	15.8	+
226 NE	1975	7	1.3	6.6	+
226 SW	1973	72.4	4.4	8.5	−
226 SW	1974	68.4	32.8	114.6	−
226 SW	1975	70	21.6	67	−
227	1972	14.4	1.2	13.2	−
227	1973	14.0	1.6	15.6	−
227	1974	14.2	2.1	19.8	−
227	1975	~6.3	0.4	6.6	+
227	1976	~6.3	1.8	9.7	+
239	1973	21	3.7	8.7	−
239	1974	23	5.0	21.5	−
239	1975	22	2.3	9.4	−
Little Otter	1971	8.8	—	—	+
Little Otter	1972	106	—	40	−

The data for the numbered lakes are all from the ELA (D. W. Schindler unpublished data) and those for Little Otter Lake are those of Michalski and Conroy (1973). For Little Otter Lake the nitrogen loadings were estimated using average DIN concentrations (dissolved inorganic nitrogen expressed as the sum of NH_3-N + NO_3^--N) and mean daily water inflows to the lake in 1972. The N loading in 1971 was not measured and therefore it has been assumed here to have been the same as in 1972. The 1971 results were obtained during a period when extremely high phosphorus input occurred from an industrial site while in 1972 this phosphorus input had been arrested.

Table 6 reveals the nitrogen fixing blue-green algae generally to be associated with waters having N:P loading ratios of less than 10. Such blue-greens are mostly absent from waters where the loading ratios are above 10. Figure 8A clearly demonstrates this relationship. The + signs indicate the presence of nitrogen fixing blue-greens while the − signs indicate the absence of these algae. The abscissa of this plot is expressed as the logarithm of the average DIN concentrations observed in the summer epilimnia. It was hypothesized that, given a sufficiently high DIN concentration, nitrogen fixation would no longer be a competitive advantage for the blue-green algae. Stripped of their advantage, it was suspected that the blue-greens would be replaced by other algae with faster growth rates. Results in Fig. 8A show that DIN concentrations apparently exerted no control over whether the blue-green fixers occurred or not. It may be that higher concentrations of DIN are required before any such effect can be noted.

Table 6 shows no clear relationship between the appearance of nitrogen fixing blue-greens and the ratio of DIN:total dissolved phosphorus (TDP). When the ratios were plotted in Fig. 8B, again as a function of summer average DIN concentrations, the scatter of points appeared random. This confirms the observation (Schindler and Fee 1975) that measurements of dissolved P and N concentrations in the ELA lakes often do not yield data indicative of loading rates. Furthermore, if the relationship between total N:total P loading ratios and the occurrence of blue-green nitrogen fixers is valid, then it follows that measurement of dissolved summer epilimnetic N and P concentrations may be of little use for prediction of such blue-green blooms.

Practical applications of these findings to water management schemes are quite clear. It appears likely that in Shield lakes or similar waters, phosphorus removal from loading would be the most desirable technique to limit eutrophication. Nitrogen removal alone is not recommended because it might lower the N:P ratio and encourage the growth of nitrogen fixing blue-green algae. These algae often form surface scums or concentrations which detract from the recreational value of lakes and they often cause taste and odor problems in drinking water. Control of such algae would obviously be important when designing and operating

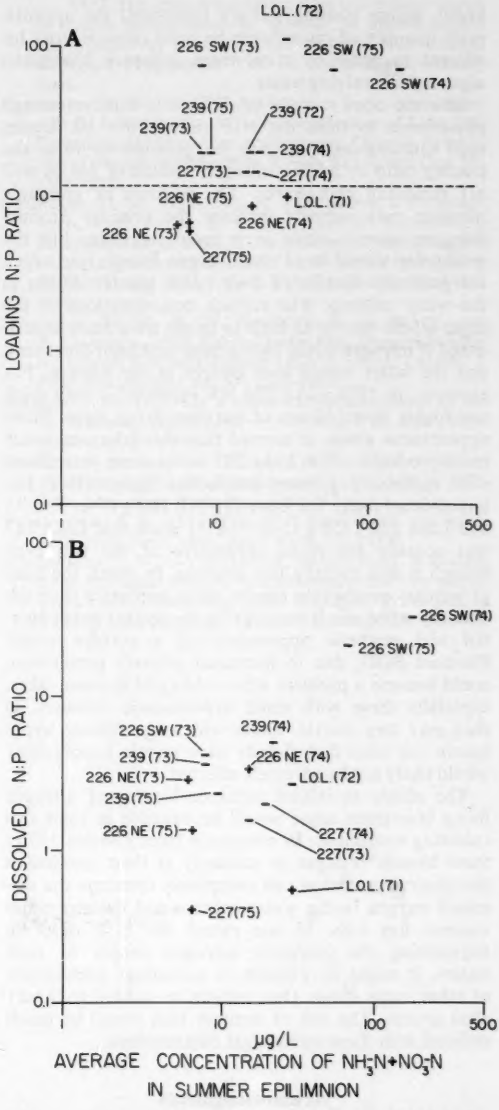

FIG. 8. A plot of + and − occurrences of blue-green fixers in a number of lakes as functions of: (A) N:P loading ratios and average dissolved nitrogen concentrations (B) dissolved inorganic N:total dissolved P ratios (summer epilimnion averaged) and dissolved inorganic nitrogen concentrations.

effluent treatment facilities. If it is valid that blue-green nitrogen fixing algae occur only when the N:P loading ratio is less than 10, then a water manager would

know, before starting tertiary treatment, the approximate quantity of phosphorus he must remove from his effluent in order to avoid these nuisance blue-green algae in his receiving water.

In some cases it could be difficult to remove enough phosphorus to raise the N:P ratio above 10. Under such circumstances it may be possible to raise the loading ratio by adding nitrogen instead of (or as well as) removing phosphorus. The addition of inorganic nitrogen may actually increase the primary production per square metre in a receiving water but the production would be of non-nitrogen fixing algae which are generally distributed over much greater depths in the water column. The surface concentrations of the algae would not be as high as might have been experienced if nitrogen fixing blue-greens had been dominant, and the water would look clearer at the surface. For example, in 1974 Lake 226 NE exhibited a very thick and highly visual bloom of nitrogen fixing algae. From appearances alone, it seemed that this lake was much more productive than Lake 227 in the same year. However, epilimnetic primary production figures (E. J. Fee unpublished data) for Lake 226 NE (82 $g\,C \cdot m^{-2} \cdot a^{-1}$) and Lake 227 (158 $g\,C \cdot m^{-2} \cdot a^{-1}$) show that Lake 227 was actually the more productive of the two even though it was visually less obvious. In short, the kind of primary production can be more important than the quantity when one is considering the recreational potential and aesthetic appearance of a surface water. Elevated BOD, due to increased primary production, could become a problem when adding N to some lakes, especially those with small hypolimnetic volumes, as they may turn anoxic. Lakes with large volume hypolimnia (or lakes that already have anoxic hypolimnia) would likely not be adversely affected.

The ability to inhibit nuisance blooms of nitrogen fixing blue-green algae would be valuable in some fish culturing operations. In numerous lakes (Barica 1975) these blooms collapse so suddenly at their conclusion that their degradation can completely consume the dissolved oxygen in the water column and thereby cause massive fish kills. If one raised the N:P ratio by augmenting the inorganic nitrogen supply to such waters, it might be possible to encourage populations of other more stable (less subject to sudden collapse) algal groups. The risk of summer kills would be much reduced with these stable algal communities.

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Denitrification in Lake 227 During Summer Stratification

Y. K. CHAN[1] AND N. E. R. CAMPBELL

Department of Microbiology, University of Manitoba, Winnipeg, Man. R3T 2N2

CHAN, Y. K., AND N. E. R. CAMPBELL. 1980. Denitrification in Lake 227 during summer stratification. Can. J. Fish. Aquat. Sci. 37: 506–512.

In situ denitrification in Lake 227 was assayed by using ^{15}N-labeled NO_3^- during summer stratification periods in 1973, 1974, and 1975. Dissolved oxygen and NO_3^- concentrations were the main factors controlling denitrification in the water column and in sediments. The epilimnetic sediment–water interface was a more significant site of natural denitrification when compared to the oxygen-limiting (dissolved oxygen less than 0.2 mg/L) thermocline and the anoxic hypolimnion. Epilimnetic sediment denitrification rates averaged about 15 mg $N \cdot m^{-2} \cdot d^{-1}$. Nearly all of the NO_3^- that mixed into the surficial sediments was denitrified. Alone, epilimnetic denitrification removed 1.4% of the NO_3^- added annually. Below the euphotic zone, sediments would provide an efficient sink for NO_3^--N without simultaneous initiation of eutrophication.

Key words: denitrification, nitrogen sink, nitrate in lakes, experimental lakes, summer stratification

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Nous avons mesuré in situ la dénitrification dans le Lac 227 en utilisant NO_3^- marqué au ^{15}N durant les périodes de stratification estivale de 1973, 1974 et 1975. Les concentrations d'oxygène dissous et de NO_3^- sont les facteurs principaux réglant la dénitrification dans la colonne d'eau et les sédiments. L'interface eau–sédiment épilimnétique est un site plus important de dénitrification naturelle par comparaison avec la thermocline et l'hypolimnion anoxique, pauvres en oxygène (moins de 0,2 mg/L d'oxygène dissous). La vitesse de dénitrification du sédiment épilimnétique est en moyenne d'environ 15 mg $N \cdot m^{-2} \cdot d^{-1}$. Presque tout le NO_3^- mélangé aux sédiments superficiels est dénitrifié. A elle seule, la dénitrification épilimnétique enlève 1,4% du NO_3^- ajouté annuellement. Au-dessous de la zone euphotique, les sédiments constitueraient une cuvette efficace pour NO_3^--N sans que soit déclenchée en même temps l'eutrophisation.

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BIOCHEMICAL removal of NO_3^--N in natural waters is achieved through two major channels, both of which are reductive processes (Hutchinson 1957). First, assimilatory reduction by green plants, mainly planktonic algae, is largely responsible for controlling the ambient NO_3^- level. Second, dissimilatory NO_3^- reduction by bacteria takes place under very low oxygen tension for the oxidation of organic and inorganic energy sources. This latter process, known as denitrification, liberates nitrogen oxides, and N_2 as the end product of NO_3^- reduction and has long been suspected as being significant in the nitrogen balance of lacustrine ecosystems (Brezonik 1977; Keeney 1973; Mortimer 1939). Review of literature (Brezonik and Lee 1968; Chen et al. 1972; Goering and Dugdale 1966; Keeney et al. 1971; Koike et al. 1972) indeed confirms the potential importance of denitrification to nitrogen budgets of lakes and sediments by using ^{15}N techniques. However, little attention has been given to direct evaluation of in situ denitrification to establish sites of significant activity on a long data base, nor have quantitative studies been conducted under varying environmental conditions that pertain in lacustrine systems by enclosing an area of relatively undisturbed sediment.

Lake 227, a small eutrophic shield lake situated in the Experimental Lakes Area (ELA), northwestern Ontario, has been intensively studied in whole-lake experiments since 1969 (Brunskill and Schindler 1971; Schindler et al. 1971, 1973; Schindler and Fee 1974). As a stably stratified lake artificially enriched with known amounts of NO_3^- and PO_4^{3-} in the summer months, Lake 227 provides a convenient model for the study of denitrification under relatively defined environmental conditions. We report here our studies on denitrification as one component of NO_3^--N removal from Lake 227 during the summer periods of 1973, 1974, and 1975 with a ^{15}N tracer technique (Chan and Campbell 1974). This technique provided for routine rate profile studies in the water column and, with slight modifications, for studies in the sediment–water inter-

[1]Present address: Department of Microbiology, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Que. H0A 1C0.

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face. Our 3-yr measurement of denitrification during the course of summer additions of fertilizer NO_3^- to Lake 227 enabled us to (a) assess the main factors affecting denitrifications; (b) determine significant sites of denitrification under natural conditions; and (c) evaluate the contribution of denitrification to NO_3^--N removal.

Materials and Methods

Water Column

Standard denitrification assays in the water column were carried out according to Chan and Campbell (1974), using 300-mL biochemical oxygen demand (BOD) bottles fitted with gas-tight rubber stoppers for incubation and the extraction of ^{15}N-labeled N_2. Triplicate water samples were collected from a specific depth at the center station of the lake by using a peristaltic pump. Samples were collected more than 24 h after addition of fertilizer NO_3^- to the lake by which time little background NO_3^- remained. To each sample, 200 μg $K^{15}NO_3$-N/L final concentration (95.0 atom %, I.C.N. Corp., Irvine, Calif.) was added by syringe immediately after collection. Treated samples were then returned to the depths from which they had been taken or to the field station for immediate incubation in the dark at temperatures within 2°C of in situ water temperatures as little difference resulted from the two methods of incubation (see below). Since N_2 production previously had been found to be linear with time in the first 2 d of incubation (unpublished results), a standard incubation time of 24 h was chosen.

After incubation, two samples from each depth of the water column were retrieved, biologically fixed by injecting 20 mg/L of $HgCl_2$ (final concentration), and stored until the time for dissolved gas extraction. Helium instead of CO_2 was used for the extraction by equilibration as a modification to the method of Chan and Campbell (1974). One of the two samples was reserved for gas chromatographic and the other for mass spectrometric analyses to determine N_2O and N_2 production respectively. Dissolved N_2O was determined by a Beckman GC72-5 gas chromatograph fitted with a helium-ionization detector and a stainless steel column (366 × 0.32 cm) packed with Porapak R (Water Assoc., Inc., Framingham, Mass.) at 55°C. Ultrapure helium was used as carrier gas at a flow rate of 20 cc/min. A standard curve was obtained by measuring peak heights for the 0–23 ng N_2O-N range of a calibration gas at 400× attenuation. The third water sample from each depth was used for NO_3^- and NO_2^- determinations at the field station. The initial in situ water temperature, dissolved oxygen (DO), NO_3^-, and NO_2^- concentration profiles were also determined (Chan and Campbell 1974). In the summer of 1973, distribution of the denitrifying bacteria in the water column was estimated by using Alexander's (1965) most-probable-number (MPN) method. The enrichment culture tubes were incubated at 22°C for 14 d.

Epilimnetic Sediment–Water System

To assay in situ denitrification at the epilimnetic sediment–water interface, a plexiglass chamber (19.8 × 19.8 × 39 cm) with an open bottom was lowered from a boat so as to rest on its winged supports at the sediment surface in a water depth of less than 2 m at a station off the northwestern shore of the lake. The chamber enclosed approximately 15 L of water and 0.04 m^2 of sediment surface. A flexible tube-loop permitted the circulation of water in the chamber overlying the sediment by a peristaltic pump operated in the boat moored above the chamber. By careful manipulation, little disturbance of the sediments was observed.

After 24 h of equilibration, 4 BOD bottles were used to take water samples from the chamber for background chemical and gaseous analyses before $K^{15}NO_3$ (95.0 atom %) was added to give a final concentration of about 500 μg N/L in the water enclosed. After 1 or 2 d of incubation, the enclosed water was circulated by pump to achieve homogeneity. Four replicate water samples were then taken in BOD bottles from the chamber. A separate set of four BOD bottles was also used to assay for denitrification in water samples collected from the water overlying the sediments external to the chamber. These samples were simultaneously incubated in situ for 48 h. From each set of four water samples, one was used for DO determination, one for NO_3^- and NO_2^- determination, and two for gaseous analyses as described above for the water column assays.

Mixing at Sediment Surface

Simultaneous measurement of the nutrient concentration of sediment interstitial water and denitrification was carried out to estimate the extent of surface sediment mixing by modifying a plexiglass chamber. A plexiglass plate was fixed vertically inside the chamber across its two opposite walls so as to form a partition from the winged-support level to the bottom of the chamber. Two rows of holes 1.5 cm apart had been drilled in the partition from its top downwards for the insertion of 8-mL plastic vials filled with distilled deionized water capped with 0.45-μm Millipore filters. When the plexiglass chamber was positioned so as to rest on its winged supports at the sediment surface, the vials were embedded in a horizontal position. Equilibration with interstitial water during the incubation period then permitted chemical analysis of the vial contents for the determination of nutrient concentration profiles in the sediment. Since the water samples in the vials were small, NO_3^- and NH_3 concentrations at each sediment depth were determined by automated methods according to Stainton et al. (1974).

Results and Discussion

Water Column

Denitrification did not occur in the epilimnion where the DO concentration was above 0.2 mg/L in summer 1973 (Fig. 1). In the anoxic hypolimnion, the rate profiles of denitrification and NO_3^- uptake followed a similar pattern. A maximum denitrification rate at 7 m was measured in June and July which reached 30 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$. The high denitrification activity correlated with the maximum MPN estimate of the denitrifying population in the anoxic waters (Fig. 1c and d). The cause of this distribution of the denitrifiers in the anoxic region was not known. The upward extension of denitrification activity to 3 m coincided with a similar extension of the anoxic region on August 1 which was due to the oxygen demand imposed by the col-

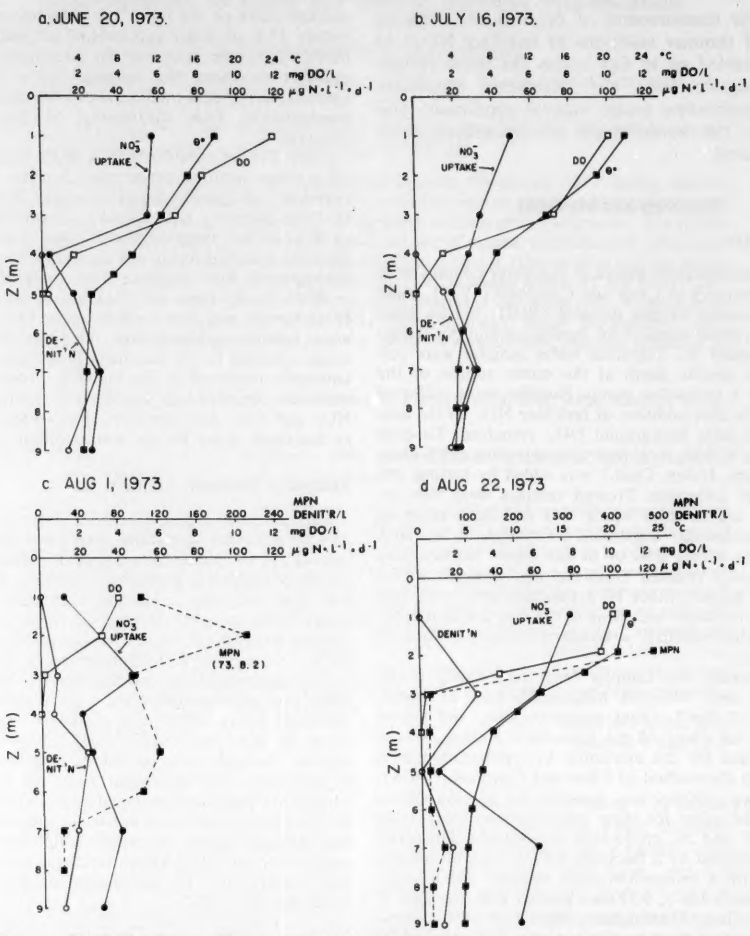

FIG. 1. Denitrification profiles and related parameters in the water column during summer 1973. An asterisk denotes detectable N_2O accumulation.

lapsed phytoplankton population (Fig. 1c). The rates of denitrification presented here represented the sum of both N_2O and N_2 production with the latter as the consistently predominant product. Nitrous oxide accumulation was not consistent. Its maximum accumulation was about 20 μg N/L after 24 h of incubation as of July 16 at 7 m (Fig. 1b) but was not significant as a denitrification product on the other occasions.

Phytoplankton were primarily responsible for NO_3^- uptake in the epilimnion. Nitrate uptake rate decreased drastically at 1 m in late July and the beginning of August as the phytoplankton died off. In late August, when a secondary algal bloom developed, NO_3^- uptake increased to about 80 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$. Because of the high rate of NO_3^- uptake, the epilimnetic NO_3^- concentration remained at about 10 μg N/L during summer despite the addition of fertilizer NO_3^-. We observed that NO_3^- did not diffuse below 5 m 2 d after each weekly addition to the lake surface before the summer of 1975. Hence, the hypolimnion was not considered to be a site of natural denitrification and this process was not measured below 5 m in 1974 and 1975.

Denitrification was again measurable only where DO dropped below 0.2 mg/L between 4.0 and 4.5 m during summer 1974 (Fig. 2). Rates of denitrification and NO_3^- uptake were comparable to those obtained in

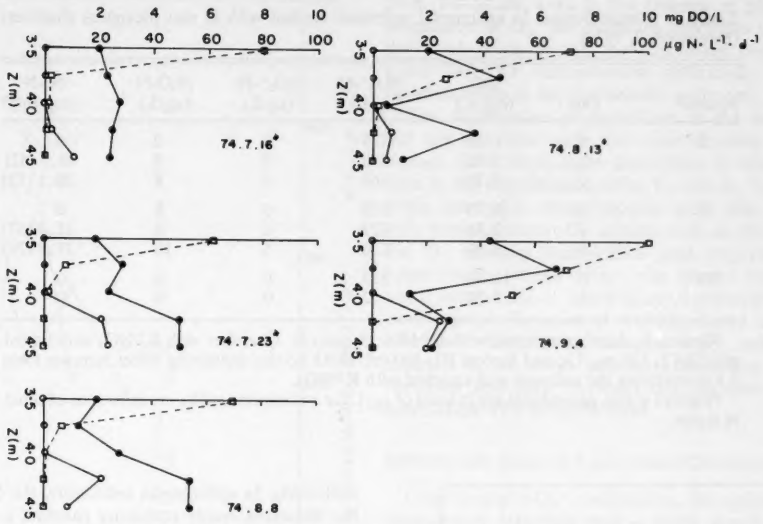

FIG. 2. Denitrification profiles and related parameters in the O_2-limiting region during summer 1974. Open circles, denitrification; closed circles, NO_3^- uptake; open squares, DO. An asterisk following the date indicates the experiment was set up just before addition of NO_3^- fertilizer to the lake.

the previous summer. Their respective ranges were 3–23 and 5–65 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$. Nitrous oxide formation was not detected.

In summer 1975, the conditions conducive to natural denitrification, namely 0.2 mg/L of DO and about 10 $\mu g/L$ of NO_3^--N, in the water column were confined to a thickness of 0.5 m in the thermocline between 3–5 m (Fig. 3). A pulse of high denitrification rate was observed in June but the average rate for the rest of the summer months was below 10 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$, reflecting the continuously low ambient NO_3^- concentration that resulted from a two-thirds reduction of NO_3^- fertilizer addition to Lake 227 that summer, i.e. from 6.3 to 2.1 $g\ N \cdot m^{-2} \cdot yr^{-1}$. Fluctuations in the uptake rate of NO_3^- might have resulted from the oscillation of the algal population in this region. Contrary to the previous summer results, N_2O formation of less than 0.2 $\mu g\ N/L$ was detected in August and September.

Epilimnetic Surficial Sediments

After the first and second day of incubation in plexiglass chambers, 19.5 and 28.1 $mg\ N_2\text{-}N/m^2$ were produced, respectively, in 1974 (Table 1). Similar results were obtained in the chamber treated with $HgCl_2$ at a rate of 20 mg/L which indicated that $HgCl_2$ was an ineffective biological inhibitor even at this high concentration for sediment systems. This observation might be explained by the strong cationic exchange capacity of sediments. The use of formaldehyde (4% final concentration) was later found to be effective, hence the denitrification activity measured in the sediments was biological. Dinitrogen was not produced in the bottled epilimnetic waters overlying the sediments, therefore the denitrification observed was effected by bacterial activity in the surficial sediments. In these and two other in situ experiments, 20% of the NO_3^- consumed in 2 d was accounted for by denitrification. The remainder was presumably taken up by phytoplankton. Nitrite accumulation was not significant. The high NO_3^- uptake rate (about 200 $\mu g \cdot L^{-1} \cdot d^{-1}$) compared to that in the epilimnion determined in the profile studies (about 60 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$) was likely due to the high enrichment used in the chamber experiment as well as to additional uptake by benthic organisms.

In contrast to the response of denitrification in the water column to the reduction of NO_3^- fertilizer loading in the summer of 1975, epilimnetic sediment denitrification rates (calculation based on 1-d incubation) remained in the same order of magnitude as those measured in the previous summer (Fig. 4). Presumably, this is due to the importance of sediment nitrification relative to NO_3^- supply from the water column. However, a dependence of NO_3^- enrichment was readily discernible when two levels of NO_3^- concentrations were used to assay denitrification on August 21. Further evidence for NO_3^- dependence was provided from the opposite trends in the magnitude of denitrification and NO_3^- uptake rates indicating that phytoplankton assimilation controlled the availability of NO_3^- to the

TABLE 1. Denitrification in epilimnetic sediments studied with in situ plexiglass chambers (September 2, 1974).

System[a]	Day	DO (mg/L)	NO_3^--N (μg/L)	NO_2^--N (μg/L)	N_2O-N (μg/L)	N_2-N (mg/m²)[b]
I	0	8.05	529	0	0	0
	1	5.95	320	0	0	19.5 (52)
	2	5.70	165	4	8	28.1 (75)
II	0	6.75	529	0	0	0
	1	3.88	329	0	0	21.4 (57)
	2	2.90	120	6	10	27.8 (74)
III	0	11.30	535	0	0	0
	2	9.20	42	0	0	0

[a]System I, chamber enriched with $K^{15}NO_3$; System II, Chamber with $K^{15}NO_3$ enrichment and $HgCl_2$ (20 mg/L); and System III, 300-mL BOD bottles containing water samples from 1.5 m overlying the sediment and enriched with $K^{15}NO_3$.

[b]Figures within parentheses are in units of μg/L for comparison with concentrations of other N forms.

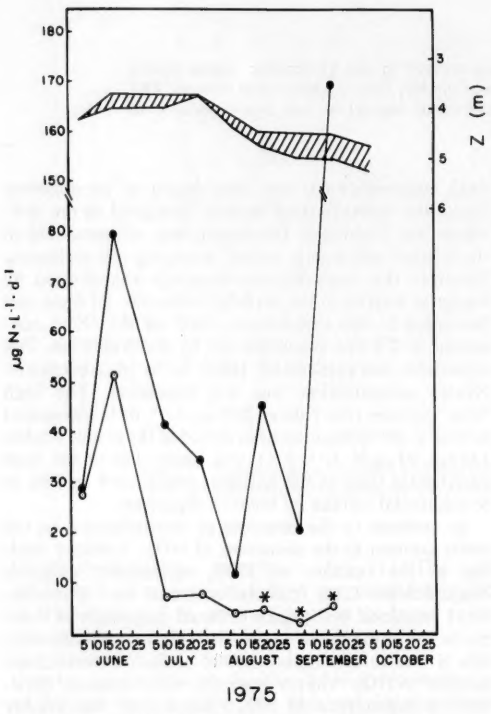

FIG. 3. Denitrification and NO_3^- uptake rates (open and closed circles, respectively) in the water column where DO was less than 0.2 mg/L and NO_3^--N was more than 10 μg/L (hatched area) during summer 1975. Asterisks denote detectable N_2O accumulation.

sediments. In epilimnetic sediments, the NO_3^- added to the sediment–water enclosure reached a depth of 2 cm in 1 d (Table 2). Together with the simultaneously determined denitrification rate and NO_3^- reduction to NH_3 in the sediments, this could serve as an estimate of the mixing rate of NO_3^- into the sediments. Since the sediments had an abundant source of NH_3 (Table 2), assimilation of NO_3^- in the sediments was considered to be unimportant during the in situ incubation. From this experiment, of the total 7.5 mg NO_3^--N added to the chamber, 5.0 mg N was found to be consumed in 1 d. About 11% of this consumption was due to denitrification and only 0.3% remained in the interstitial water. The NO_3^- mixing rate into the epilimnetic sediments undre the experimental conditions was then 13.7 mg $N \cdot m^{-2} \cdot d^{-1}$, which was essentially equivalent to the denitrification rate. It implied that nearly all the NO_3^- that mixed into the sediments was denitrified. The mechanism of natural mixing was, in the absence of the chamber, probably a combination of diffusion, wind action, and turbulence. Mixing by CH_4 ebullition was not apparent at the site studied.

FACTORS AFFECTING DENITRIFICATION RATES

It is evident from the above that DO was the pri-

TABLE 2. Interstitial NO_3^- and NH_3 gradients in epilimnetic sediments of Lake 227 one day after NO_3 addition to the enclosed sediment–water system (July 30, 1975).

Depth (cm)	NO_3^--N (μg/L)	NH_3-N (μg/L)
1.5	22	145
3.0	6	254
4.5	5	313

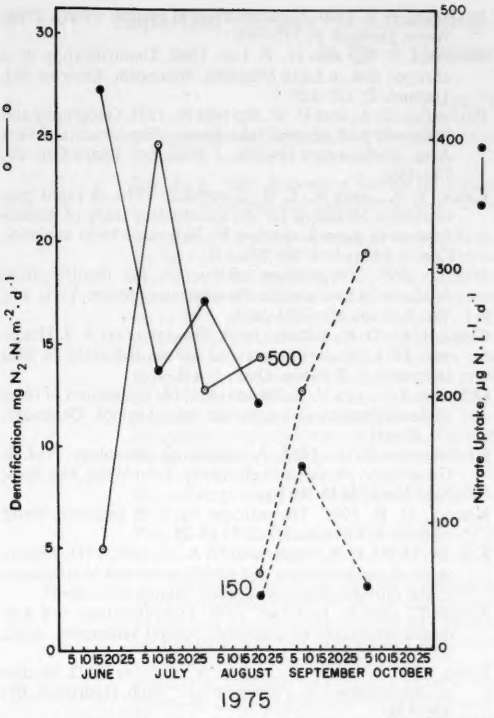
Denitrification, mg $N_2 \cdot N \cdot m^{-2} \cdot d^{-1}$
Nitrate Uptake, $\mu g \cdot N \cdot L^{-1} \cdot d^{-1}$
500
150
5 10 15 20 25 5 10 15 20 25 5 10 15 20 25 5 10 15 20 25 5 10 15 20 25
JUNE JULY AUGUST SEPTEMBER OCTOBER
1975

FIG. 4. Denitrification in the epilimnetic sediment–water interface during summer 1975. On August 21, both 150 and 500 μg NO_3^--N/L enrichments were used. The high enrichment was used before, and the low after August 21.

mary controlling factor of denitrification. The existence of microaerobic or anaerobic conditions was inferred at the epilimnetic sediment–water interface to permit biological denitrification. Second to DO, the availability of NO_3^- was a controlling factor of denitrification in both water column and epilimnetic sediments. The dependence of denitrification on NO_3^- concentration was also directly demonstrated in anoxic hypolimnetic water samples by addition of various amounts of $^{15}NO_3^-$ up to 1.5 mg N/L. The saturating concentration and the apparent half-saturation constant were about 400–150 μg NO_3^--N/L, respectively.

Light had little effect on denitrification in the water column and epilimnetic sediments. Similar denitrification rates were obtained for anoxic hypolimnetic waters incubated either in situ or at in situ temperature in the dark. Rates of sediment denitrification measured with one clear and one light-proof chamber were also similar (about 15 mg $N \cdot m^{-2} \cdot d^{-1}$) although the DO concentration decreased from 7.2 mg/L to 5.5 and 3.1 mg/L in the clear and dark chambers respectively in 24 h. Therefore, even in the absence of photosynthetic generation of O_2, the oxidative processes (respiration) that took place during the incubation period did not lower the DO concentration sufficiently to promote denitrification in the epilimnetic sediments.

The distribution of denitrifiers in the water column did not correlate with the denitrification profile. Although there were more denitrifiers in the epilimnion than in the hypolimnion (Fig. 1c and d), being facultatively anaerobic, these bacteria were able to denitrify only under reduced O_2 tension such as that existing in the O_2 limiting thermocline and hypolimnion. The hypolimnion, where NO_3^- was absent, however, was not a natural site of denitrification although denitrifiers were present. Koike et al. (1972) found in a brackish lake that denitrifying bacteria were uniformly distributed in the water column irrespective of seasonal variation and their numbers did not show a positive correlation with denitrification.

Significant Sites of Lacustrine Denitrification

Considering NO_3^- availability, the epilimnetic sediment–water interface was a more significant site of natural denitrification than the oxic–anoxic boundary and the anoxic hypolimnion. The bottom waters would be more significant in early summer when ambient NO_3^- concentration remains high and DO gradually decreases as stratification proceeds. On the other hand, the surface-applied NO_3^- that reached the epilimnetic sediments added to conditions conducive to denitrification at the sediment–water interface. Recently, Sørensen (1978) demonstrated in a coastal marine sediment that denitrification decreased rapidly with sediment depth where NO_3^- concentration also decreased below the sediment surface. In the sediment–water system, the relative importance of denitrification was likely determined by its competition with assimilation by plants and sediment immobilization for NO_3^-. This inorganic form of N could be provided by artificial input and natural processes such as runoff, precipitation, and nitrification.

Importance of Denitrification to the Nitrogen Budget

We estimated that epilimnetic sediment denitrification could remove 1.4% of the total addition of NO_3^- fertilizer (about 300 kg N) in Lake 227 during summer stratification. This estimation was based on the known bathymetry of the lake (Brunskill and Schindler 1971) assuming an average in situ denitrification rate of 15 mg $N \cdot m^{-2} \cdot d^{-1}$. Although sediment denitrification as a nitrogen sink was small compared to the fast NO_3^- uptake by phytoplankton, it may serve as a significant sink for NO_3^--N under anoxic conditions during early stratification and phytoplankton collapse. It may also be important when fall overturn is coincident with or

closely followed by early freeze-up when CH_4 oxidation depletes DO (Rudd and Hamilton 1978). Investigations of such processes and their effect on the annual contribution of denitrification to nitrogen removal are required. Denitrification in the oxic–anoxic thermocline is not a significant sink for NO_3^--N under the ambient condition studied because of overestimation due to addition of tracer NO_3^- at best it can be regarded only as a potential sink. As pointed out by Koike et al. (1972), it becomes more difficult, if not impossible, to measure the absolute in situ denitrification rate since the addition of tracer NO_3^- inevitably raises the level of in situ NO_3^-. On the other hand, low enrichment could result in N_2 production beyond the sensitivity of detection (0.3 μg N_2/L by mass spectrometry in our studies). Before 1975, each weekly addition of NO_3^- to Lake 227 surface produced a high NO_3^- concentration (more than 100 μg N/L) in the epilimnion which lasted for about 5 h (unpublished data). During this transient period substrate limitation for sediment denitrification was believed to be nonexistent and overestimation of natural denitrification was not anticipated in the relatively short-term incubation (24 h) enclosure studies.

The production of N_2O was probably not important in our present studies. In the sediment–water system, N_2O formation was only associated with the high NO_3^- enrichment (500 μg N/L). In pure cultures of denitrifiers isolated from the lake, N_2O production was temperature as well as species dependent (Chan and Campbell 1975).

More in situ denitrification studies at the sediment surface in the presence of natural vegetation (algae and macrophyte) in relation to NO_3^- concentration are warranted in order to elaborate the nitrogen budgets of lakes. To date, only one other lacustrine study of in situ sediment denitrification had been reported and unusually high rates (60–80 mg $N \cdot m^{-2} \cdot d^{-1}$) were found (Tirén et al. 1976). Since recent reports showed the existence of a potential NO_3^- reduction to NH_3 in anaerobic marine sediments (Koike and Hattori 1978; Sørensen 1978), this aspect of NO_3^- reduction should also be included in future studies.

Acknowledgments

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Important Factors for Estimating Annual Phytoplankton Production in the Experimental Lakes Area

EVERETT J. FEE

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

FEE, E. J. 1980. Important factors for estimating annual phytoplankton production in the Experimental Lakes Area. Can. J. Fish. Aquat. Sci. 37: 513–522.

Annual phytoplankton growth rates from 21 lakes in the Experimental Lakes Area (ELA) for the 4-yr period 1973–76 are reported. The coefficient of variation of production in most lakes over this period was about 20%. This variability was the same for both control and experimental lakes. Subthermocline populations were quantitatively unimportant in eutrophied basins but up to 50% of annual production occurred in hypolimnion chlorophyll peaks in some transparent control lakes. The exact details of chlorophyll distribution as monitored with an in vivo fluorometer are unnecessary for accurate measurements of production, even in lakes having hypolimnion chlorophyll peaks. Annual production rates were about 80% of the rates predicted with simulated cloudless weather. The common practice of not correcting production estimates for basin morphometry when reporting areal production rates resulted in overestimation of productivity by roughly 20%. The results indicate several ways for simplifying the numerical primary production model.

Key words: production, morphometry, models, limnology, Experimental Lakes Area, incubator

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Nous donnons ici les taux de croissance annuels du phytoplancton de 21 lacs de la région des lacs expérimentaux (ELA) sur une période de 4 ans, 1973–76. Le coefficient de variation de production dans la plupart de ces lacs durant cette période fut d'environ 20%. La variabilité est identique à la fois dans les lacs témoins et les lacs expérimentaux. Les populations de la sous-thermocline sont quantitativement peu importantes dans les bassin eutrophisés, mais dans certains lacs témoins transparents, jusqu'à 50% de la production annuelle se fait en périodes de pointe de chlorophylle dans l'hypolimnion. Il n'est pas nécessaire, pour des mesures précises de production, de connaître les détails de la répartition de la chlorophylle telle que mesurée de façon continue par un fluoro mètre *in vivo*, même dans les lacs qui ont des pics de chlorophylle dans l'hypolimnion. Les taux de production annuels sont environ 80% des taux prédits par temps sans nuages simulé. Le manque à corriger les estimations de production pour tenir compte de la morphométrie du bassin, une pratique commune, quand il s'agit de rapporter les taux de production par superficie entraîne une surestimation de productivité d'environ 20%. Ces résultats nous éclairent sur plusieurs manières de simplifier le modèle numérique de production primaire.

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FROM 1973 to 1976 I estimated annual primary production from control and experimental basins in the Experimental Lakes Area using an incubator technique (Fee 1977). I have already used some of these data to analyze the relation between lake morphometry and productivity, expressed per unit volume of epilimnion (Fee 1979), and the seasonal patterns of primary production (E. J. Fee unpublished data). In this paper I summarize the annual rates calculated in various ways. These data are used to assess the quantitative importance of several components of the photosynthesis model. Based on these results, simplified procedures for estimating annual primary production with the incubator method are given. The importance of correcting production estimates for the diminution of lake volume with depth is assessed also.

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Methods

As the incubator method is described in detail elsewhere (Fee 1973, 1977) only a brief summary of the technique is needed here. Three primary variables are monitored as input to the computer model: 1) the distribution over time of irradiance at the water surface, 2) the extinction of light as a function of depth in the lake, and 3) the photosynthetic response of a unit volume of water to a range of irradiances. The surface irradiances were continuously monitored and digitized at 5-min intervals over the entire time interval for which calculations were to be made. For each value of surface irradiance the absolute irradiance at

each depth was calculated from the light extinction data. The photosynthesis vs. irradiance curve applicable to each depth was then used to calculate the instantaneous production rate profile from the irradiance profile. This procedure was repeated for all surface irradiance data for the day. The instantaneous rates for each depth were integrated over time to give a depth profile of the total daily production. One advantage of this approach over direct in situ experimentation is that since the components of the system are separately measured, production can be simulated for any assumed as well as actual environmental conditions. Thus it is possible not only to estimate actual annual primary production but, in addition, simulated cloudless surface irradiance can be used to remove year-to-year variance due to annual variations in cloudiness.

Light is the variable that links the three main components of the photosynthesis model. In 1973 Instrumentation Specialties Co. (ISCO) scanning spectroradiometer records were integrated from 400 to 700 nm to give the total light energy available for photosynthesis in the incubator. In subsequent years the photosynthetically more meaningful unit of total light quanta between 400 and 700 nm was adopted and incubator irradiances were measured with a Lambda Instruments Co. model LI-192 quantum cell. The quantitative difference between measurements made with the two instruments is unknown. However, the year-to-year similarity of annual production figures for any specific lake (see below) shows that this is a minor source of variance and that either technique is suitable.

Light extinction in the lakes was measured with a Whitney–Montedoro cadmium sulfide cell in 1973 and with the Lambda quantum cell in 1974 through 1976. Mean extinction coefficients in control lakes were virtually identical in the years when different instruments were being used which shows that the different spectral sensitivities of the instruments had little effect on the parameter that was measured. Measurements were made in 1978 on one of the most eutrophic basins (L226 NE) to determine the error associated with the 2-wk sampling interval. During a 2-wk period in mid June when the extinction coefficient changed from 0.75 to 0.97 m^{-1} due to the development of a massive algal bloom the estimate of total production made with weekly transparency measurements agreed to within 1% of the value obtained with the 2-wk sampling interval. Since transparency changes during any 2-wk period are much smaller in all other basins it is clear that the 2-wk sampling interval introduces little error.

Surface irradiances were measured with an Eppley precision pyranometer in 1973 and 1974 and with a Lambda quantum cell in 1975 and 1976. The pyranometer was calibrated with a quantum cell in 1974 and all previous data have been converted to quantum units. Cloudless weather was simulated by fitting a truncated cosine curve to data from cloudless days recorded at ELA. Fee (1977) outlines the procedure.

The basic photosynthesis model described above was extended to include secondary phenomena that influence model estimates. First, the pattern of diurnal variation of photosynthetic rates under identical incubation conditions over a day was established so that samples taken in the morning could be extrapolated to estimate daily rates (Fee 1975).

The method of obtaining water samples for ^{14}C uptake incubation presented a more difficult problem. In 1973 samples were taken with an opaque Van Dorn bottle at two discrete depths in each stratum (epilimnion, metalimnion, and hypolimnion). For the simulation of in situ production the photosynthetic rates were applied to a range of depths; for example, the photosynthetic rates from a sample taken at 5 m might have been used to estimate production at depths from 4 to 7 m. In 1974 narrow bands of high chlorophyll concentration were discovered in the metalimnion and hypolimnion of certain ELA lakes (Fee 1976). Estimates of integral primary production from discrete hypolimnion samples were highly variable as a result. This variance was eliminated by sampling with an integrating water bottle and this sampling technique was used for the duration of the study at all depths starting in early August of 1974. In lakes exhibiting large hypolimnion blooms, estimates of areal primary production (uncorrected for morphometry) increased by two to three times when this sampling procedure was adopted. The high rates persisted until vertical mixing in early September destroyed the apparently highly productive hypolimnion bloom.

In contrast with the high production rates obtained in the model, independent estimates of phytoplankton growth rates in the hypolimnion derived from weekly in vivo fluorometer profiles were low (Fee et al. 1977; Fee 1978a). To resolve the discrepancy between these conflicting results, a series of in situ and model comparisons were made in 1976 (Fee 1978b). This investigation showed that the model overestimated hypolimnion production rates by two to five times. Differences in the spectral composition of light between the lake and the incubator were shown to be responsible for the error. For this reason, a light quality correction was introduced into the model. All of the results reported in this paper have been corrected for this light quality problem.

Finally, in 1976 the model was modified to include any degree of detail in the description of the vertical distribution of chlorophyll. This was done by dividing the incubator production rates by the chlorophyll concentration of the incubated sample. In situ production rates were then calculated by multiplying the normalized incubator rate by the chlorophyll concentration at each depth as measured with an in vivo fluorometer system. This procedure assumes that the assimilation number (P_{opt}/Chl) is constant within each stratum of the lake at any given time.

A step-by-step description of the calculation procedure as well as a listing of the computer program is given by Fee (1977).

Data

Table 1 summarizes the number of measurements of primary production and transparency made in the ELA lakes during the study period. For incubator measurements three separate strata from each lake (epilimnion, metalimnion, and hypolimnion) were sampled once every week. In 1973 two Van Dorn bottle samples were taken at discrete depths from each stratum; in 1974 one such sample was taken from each stratum until August, when integrated sampling was initiated. Transparency measurements were made once every 2 wk. Details of the laboratory and field methods along with a complete presentation of the raw data have been published in the Technical and Data Report Series of the Fisheries and Marine Service (Incubator data: Shearer and Fee 1974; Shearer 1976a; DeClercq and Shearer 1976; DeClercq et al. 1977; Transparency data: Reid et al. 1975a; Shearer 1976b; Shearer

and DeClercq 1976, 1977). The chlorophyll profiles used for the 1976 calculations are given by Fee et al. (1977). The continuous strip chart records of surface irradiance were digitized with a mechanical pen follower. The computer program in Fee (1971) was used to convert these raw insolation data to 5-min average values. These data are on file at the Freshwater Institute, Winnipeg.

Fee (1979) has summarized general information on the size, depth, transparency, thermal regime, and nutrient loadings for the study lakes. Detailed morphometric maps of the ELA lakes are on file at the Freshwater Institute.

The annual primary production data are presented in Table 2. The lakes have been divided into two main groups: control and experimental. The lakes are further divided according to the presence or absence of thermal stratification. Finally, the three very clear oligotrophic control lakes are separated from the others which have visibly brown water.

To include production in hypolimnion chlorophyll peaks which are normally at the depth where 1% of surface light occurs (Fee 1976), the euphotic zone was defined as extending from the surface to that depth where 0.5% of surface light occurred. The epilimnion depth was taken as the depth of the mixed layer in early August. Calculations were made for the ice-free season — about May 1 to November 1.

Twelve different production totals are listed. The first six refer to production in a column of water at the center of the lake; most data in the literature have been calculated in this manner. The last six are corrected for the diminution of volume with depth. Finite difference approximations of the following intergral equations were used for the calculations:

Column in Table 2	Equation
1	$\int_0^{z_e} P(z)\,dz$
2	$\int_0^{z_e} P(z)\,dz/z_e$
3	$\int_0^{z_{eu}} P(z)\,dz$
4	$\int_0^{z_{eu}} P(z)\,dz/z_{eu}$
5	$\int_0^{z_{eu}} P_c(z)\,dz$
6	$\int_0^{z_{eu}} P_c(z)\,dz/z_{eu}$
7	$\int_0^{z_e} P(z)\,A(z)\,dz/A_o$
8	$\int_0^{z_e} P(z)\,A(z)\,dz/V_e$
9	$\int_0^{z_{eu}} P(z)\,A(z)\,dz/A_o$
10	$\int_0^{z_{eu}} P(z)\,A(z)\,dz/V_{eu}$
11	$\int_0^{z_{eu}} P_c(z)\,A(z)\,dz/A_o$
12	$\int_0^{z_{eu}} P_c(z)\,A(z)\,dz/V_{eu}$

where $P(z)$ is the rate of primary production as a function of depth,
$P_c(z)$ is primary production as a function of depth calculated with simulated cloudless weather,
$A(z)$ is the area of a plane surface at depth z,
z_{eu} is the depth of the euphotic zone,
z_e is the depth of the epilimnion,
A_o is the surface area of the lake,
V_{eu} is the volume of the euphotic zone,
and V_e is the volume of the epilimnion.

Results

Areal primary production rates uncorrected for lake morphometry overestimated morphometry corrected primary production by 16–17% (Fig. 1). Highest percentage errors occurred in the smallest lakes while in the largest lake (L228, $A_o = 1672$ ha) the error was only 6%. The error was almost identical for both euphotic zone and epilimnion estimates. When the data were expressed on a volumetric basis (Fig. 2) the error changed sign, i.e. uncorrected estimates were less than the morphometry corrected values. In this case, however, the error for the whole euphotic zone was 17% while for the epilimnion alone it was only 4%. Further, the scatter of the data was much lower for the epilimnion than for the whole euphotic zone.

For 2 yr, the ELA primary production work focused on the development of accurate methods for the study of subepilimnion algal populations (Fee 1973, 1976, 1977). It is relevant, therefore, to inquire about the importance of these populations to annual primary production. The regression result (Fig. 3) implies that on average epilimnion production was 95–97% of the euphotic zone figure. This is a biased way of looking at the data, however, since the higher values of production in eutrophic basins dominate this result. Epilimnion production would be expected to be a higher percentage of the total production in these basins since nutrients were added to surface waters. In the oligotrophic control lakes epilimnion production varied from 46 to 92% of the annual total, with a median value of 76%. As expected, in the experimentally

TABLE 1. Summary of the number of data sets used for the annual primary production calculations. "Inc." refers to incubator measurements of primary production, "Transp." refers to measurements of transparency.

	1973		1974		1975		1976	
Lake	Inc.	Transp.	Inc.	Transp.	Inc.	Transp.	Inc.	Transp.
114			17	11	18	15		
120			22	10				
223			25	11	27	13	33	12
224			25	11	27	11	33	13
226NE	40	15	38	13	32	15	33	29
226SW	32	14	32	13	34	15	33	28
227	30	15	27	12	27	14	27	15
228			27	14				
230					33	20	21	11
239	49	14	52	12	30	11	29	15
240			35	13	29	15		
261	22	13	28	13	28	14	26	14
302N	34	14	42	15	35	13	30	24
302S	35	14	42	14	46	13	30	27
303			15	7	18	15	14	12
304	28	13	32	10	27	15	26	14
305			29	12				
383			25	14	27	18		
470					19	9		
Totals	240	112	513	205	457	212	335	191

TABLE 2. ELA annual phytoplankton primary production rates calculated in the several ways described in the text. Odd numbered columns have the units of g $C \cdot m^{-2} \cdot yr^{-1}$, even numbered columns are in units of g $C \cdot m^{-3} \cdot yr^{-1}$.

		Uncorrected for morphometry						Corrected for morphometry					
		Actual weather				Cloudless		Actual weather				Cloudless	
		Epilimnion		Euphotic Zone				Epilimnion		Euphotic Zone			
Lake	Year	1	2	3	4	5	6	7	8	9	10	11	12
Control lakes													
Stratified lakes													
Clear lakes (0.2 < extinction coefficient < 0.4)													
224	74	12.8	2.6	23.8	1.3	29.5	1.6	11.1	2.6	17.7	1.6	21.1	1.9
	75	8.9	1.6	22.6	1.3	26.7	1.5	7.7	1.7	15.5	1.4	17.7	1.7
	76	14.0	1.9	21.5	1.2	26.3	1.5	11.5	2.0	15.3	1.4	18.3	1.7
228	74	17.9	1.8	22.4	1.1	27.3	1.4	16.9	1.8	20.8	1.2	25.1	1.4
305	74	15.8	2.6	23.6	1.2	28.9	1.4	13.7	2.6	19.2	1.5	23.1	1.8
Colored lakes (0.4 < extinction coefficient)													
120	74	8.9	3.6	17.7	2.2	21.6	2.7	8.2	3.6	14.9	2.2	17.8	2.6
223	74	14.6	4.1	39.1	3.9	49.5	5.0	12.9	4.1	28.0	3.9	34.3	4.8
	75	13.4	2.7	23.0	2.3	27.5	2.8	11.0	2.6	16.5	2.5	19.1	2.8
239	73	16.1	4.0	18.2	2.3	25.0	3.1	14.2	4.1	15.8	2.6	21.2	3.4
	74	16.1	4.0	19.9	2.5	24.1	3.0	14.3	4.1	17.1	2.5	20.4	2.9
	75	16.2	3.4	18.3	2.3	21.3	2.7	14.1	3.5	15.6	2.5	17.9	2.9
	76	16.9	4.2	21.1	2.6	25.9	3.2	14.8	4.2	17.8	2.9	21.6	3.5
240	74	16.9	4.2	23.0	2.3	28.0	2.8	14.1	4.3	17.8	3.0	21.0	3.6
	75	13.9	2.9	16.5	2.1	19.1	2.4	11.4	3.0	12.8	2.4	14.4	2.7
383	74	26.0	8.2	31.5	5.3	39.2	6.5	20.2	7.8	23.6	5.5	28.7	6.7
	75	16.0	5.3	26.7	4.5	32.3	5.4	12.8	5.4	19.0	4.8	22.3	5.6
Unstratified lakes													
114	74	41.4	11.0	41.4	11.0	51.3	13.6	23.7	13.5	23.7	13.5	26.0	14.9
	75	38.7	10.3	38.7	10.3	43.7	11.6	19.8	11.3	19.8	11.3	21.1	12.0
303	74	37.4	19.0	37.4	19.0	45.0	22.5	30.6	20.7	30.6	20.7	33.1	22.4
470	75	9.7	6.5	9.7	6.5	10.9	7.3	5.5	7.0	5.5	7.0	6.0	7.5

TABLE 2 (*Concluded*)

		Uncorrected for morphometry						Corrected for morphometry					
		Actual weather				Cloudless		Actual weather				Cloudless	
		Epilimnion		Euphotic Zone				Epilimnion		Euphotic Zone			
Lake	Year	1	2	3	4	5	6	7	8	9	10	11	12
Experimental lakes													
Stratified lakes													
223	76	19.3	3.9	34.0	3.4	41.3	4.1	16.3	3.9	24.0	3.9	28.6	4.3
226SW	73	43.0	10.8	58.8	7.4	78.6	9.8	36.8	10.8	46.1	8.2	59.6	10.6
	74	37.0	9.3	50.2	6.3	60.8	7.6	31.8	9.4	39.8	6.4	46.9	7.6
	75	37.0	9.3	57.5	7.2	68.4	8.6	31.6	9.3	44.1	7.8	51.3	9.1
	76	32.8	8.2	44.1	5.5	53.8	6.7	28.3	8.3	34.9	6.2	41.9	7.4
226NE	73	60.9	15.2	72.1	9.0	96.5	12.0	52.4	15.9	57.2	11.8	73.6	15.2
	74	81.9	20.5	89.0	11.1	105.9	13.2	71.5	21.7	74.9	14.2	87.0	16.5
	75	63.5	15.9	74.3	9.3	86.1	10.8	54.2	16.4	59.1	12.2	66.7	13.8
	76	89.0	22.3	98.0	12.3	117.0	14.6	76.5	23.2	80.5	16.6	95.0	19.6
227	73	188.5	94.3	204.7	40.9	267.1	53.4	161.8	97.2	172.0	51.9	217.8	65.7
	74	158.0	63.2	164.5	32.9	196.0	39.2	131.4	66.3	135.5	40.9	158.0	47.7
	75	43.7	17.5	56.1	11.2	65.8	13.1	35.6	18.0	42.8	12.9	48.9	14.8
	76	75.5	37.8	103.6	20.7	124.9	25.0	63.2	38.0	79.5	24.0	94.3	28.5
302S	73	36.0	9.0	48.0	6.0	65.0	8.1	30.0	9.0	36.2	7.2	47.0	9.4
	74	25.8	7.1	49.4	6.2	61.1	7.7	22.1	7.2	35.1	6.9	41.8	8.2
	75	22.7	4.7	29.9	3.7	35.0	4.3	18.5	4.8	21.8	4.4	24.5	4.9
	76	28.5	7.1	42.7	5.3	51.1	6.3	23.8	7.2	30.2	6.0	35.2	7.0
302N	73	39.5	9.9	49.7	6.2	66.4	8.3	32.0	10.1	37.2	7.4	48.0	9.5
	74	34.8	9.6	61.7	7.7	77.1	9.6	28.9	9.8	43.2	7.8	52.1	9.4
	75	35.1	7.3	41.8	5.2	49.5	6.2	27.5	7.6	30.9	6.1	35.5	7.0
	76	47.9	12.0	60.6	7.6	72.8	9.1	38.6	12.2	45.2	8.9	53.3	10.5
304	73	57.9	23.2	67.2	13.4	88.8	17.8	48.9	24.0	53.4	17.7	67.5	22.4
	74	39.4	15.8	54.9	11.0	65.4	13.1	32.5	15.9	39.7	13.2	45.7	15.2
	75	82.5	27.5	87.4	17.5	102.0	20.4	67.8	29.2	70.0	23.2	76.7	25.4
	76	59.8	23.9	70.2	14.0	83.6	16.7	50.3	24.7	55.3	18.4	64.6	21.5
261	73	37.7	12.6	41.0	6.8	56.6	9.4	26.9	13.3	28.0	10.9	36.5	14.2
	74	37.8	15.8	48.1	8.0	59.4	9.9	28.5	16.1	32.4	12.0	38.3	14.1
	75	26.4	8.8	30.1	5.0	35.9	6.0	19.1	9.5	20.4	7.9	23.3	9.0
	76	30.2	10.1	34.9	5.8	42.8	7.1	22.1	10.5	23.6	9.2	28.1	10.9
230	75	21.1	6.6	27.7	4.6	32.6	5.4	17.8	6.5	22.1	4.2	25.5	4.9
	76	22.7	11.4	38.0	6.3	45.5	7.6	20.5	11.3	31.2	8.1	36.8	9.5
Unstratified lake													
303	75	58.4	29.2	58.4	29.2	64.2	32.1	44.6	30.2	44.6	30.2	48.1	32.6
	76	79.9	40.0	79.9	40.0	92.9	46.5	61.3	41.5	61.3	41.5	70.3	47.6

eutrophied basins most values of the ratio are greater than 90%. Low percentage of epilimnion production occurred in experimental lakes with hypolimnion or winter fertilization (L302 N, L230) or in lakes that did not receive epilimnion additions of phosphorus (L223, L226 SW, L304 in 1973 and 1974, L302 S). To conclude, in thermally stratified basins where appreciable light penetrates into the hypolimnion, up to 50% of annual production occurred in subepilimnion populations and these must be studied to obtain accurate annual production figures. In most eutrophic lakes the mixed zone is deeper than the euphotic zone and the production in subepilimnion populations can be ignored.

On average, production estimates obtained with actual solar irradiance were 80% of the values obtained with simulated cloudless surface irradiance (Fig. 4). However, this regression result with annual data from all lakes and all years masks important lake-to-lake, daily, and annual variability. In the shallow basins (L114 and L303) where appreciable quantities of light reach the sediments actual production averaged 91% of that given by the cloudless model. On a daily basis the percentage in a single lake varied from 15 to 105% (E. J. Fee unpublished data). That is, production on any specific day could not be reliably predicted from the result obtained with the cloudless model of insolation. Complete data are available for eight lakes with which to assess the yearly variability of this figure (Table 3). In 1973 it was exceptionally cloudy and actual annual production averaged 78% of the figure

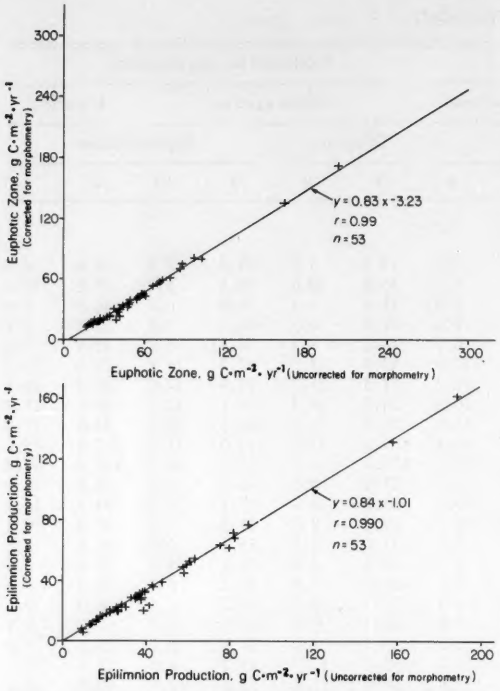
Euphotic Zone, g C·m⁻²·yr⁻¹ (Corrected for morphometry)
y = 0.83 x - 3.23
r = 0.99
n = 53
Euphotic Zone, g C·m⁻²·yr⁻¹ (Uncorrected for morphometry)
Epilimnion Production, g C·m⁻²·yr⁻¹ (Corrected for morphometry)
y = 0.84 x - 1.01
r = 0.990
n = 53
Epilimnion Production, g C·m⁻²·yr⁻¹ (Uncorrected for morphometry)

FIG. 1. The relation between areal primary production of a water column at the center of the lake (x axis) and areal production corrected for the diminution of volume with depth (y axis) in ELA lakes.

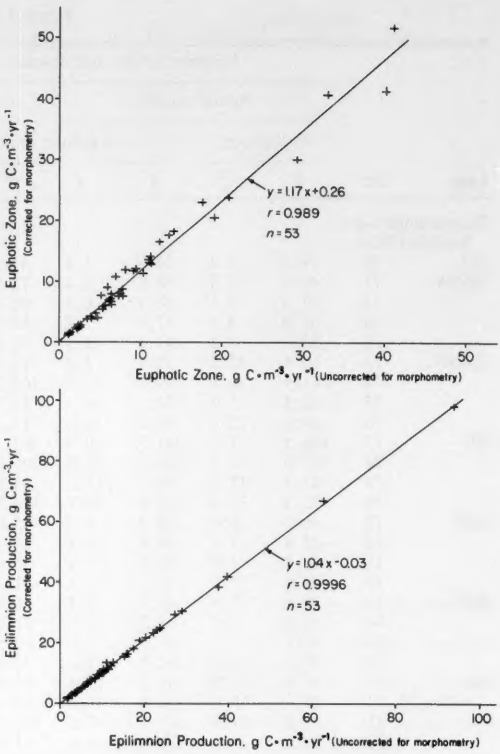
Euphotic Zone, g C·m⁻³·yr⁻¹ (Corrected for morphometry)
y = 1.17 x + 0.26
r = 0.989
n = 53
Euphotic Zone, g C·m⁻³·yr⁻¹ (Uncorrected for morphometry)
Epilimnion Production, g C·m⁻³·yr⁻¹ (Corrected for morphometry)
y = 1.04 x - 0.03
r = 0.9996
n = 53
Epilimnion Production, g C·m⁻³·yr⁻¹ (Uncorrected for morphometry)

FIG. 2. The relation between primary production in ELA lakes expressed per unit volume of water uncorrected and corrected for the morphometry of the lake. Separate figures are shown for the euphotic zone and epilimnion.

given by the cloudless model. In the other 3 yr this ratio varied only from 85 to 87% and showed no relation to actual variations of insolation. The conclusion is that normal annual variations of cloudiness are responsible for little year-to-year variance in productivity.

By using simulated cloudless weather all of the year-to-year variance due to cloudiness has been removed. This allows us to use the ELA productivity data to approach the question "what is the year-to-year variability of annual production in natural ecosystems with the influence of cloudiness removed?"

The variance of annual productivity has been expressed as the coefficient of variation (standard deviation/mean) to allow data of different magnitudes to be compared. These ratios have been calculated separately for the euphotic zone and the epilimnion (Table 4). All calculations were made with morphometry corrected estimates. In general, the standard deviation of annual areal production was about 20% of the mean in both the euphotic zone and the epilimnion. Some basins were remarkably better than this (L224, L239) and some remarkably worse (L223, L227). The fact that the lowest variability occurred in lakes with the longest residence times may not be purely coincidental. Fee (1979) discusses possible causes of the unusually high variability of production in L227. The high variability in L223 was due to the presence or absence of hypolimnion blooms (Fee 1976) since the variability of the epilimnion data alone was only 15% of the mean (Table 4).

The variability of production in unstratified lakes was not consistently higher or lower than in thermally stratified basins. Excluding L227, the experimentally eutrophied basins did not exhibit more variability than control basins. The 20% figure therefore is the demonstrated precision of the incubator method for estimating annual primary production. Whether this is methodological variance or variance inherent in the ecosystems is unknown.

The importance of refining the model by incorporating exact details of vertical distribution of chlorophyll as recorded with an in vivo fluorometer can be assessed by comparing the results obtained in 1976—

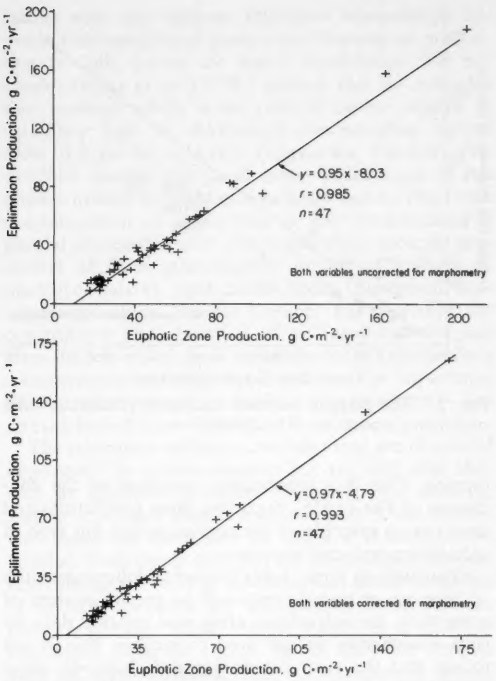
Epilimnion Production, g C·m⁻²·yr⁻¹
Euphotic Zone Production, g C·m⁻²·yr⁻¹
y = 0.95x - 8.03
r = 0.985
n = 47
Both variables uncorrected for morphometry
y = 0.97x - 4.79
r = 0.993
n = 47
Both variables corrected for morphometry

FIG. 3. The relation between euphotic zone and epilimnion areal primary production before (above) and after (below) correction for lake morphometry.

when this was done — with results from previous years. In all lakes where four years of data are available there is little difference (Table 2). It is apparent that this procedure is an unnecessary complication.

Finally, Fig. 5 demonstrates a highly variable relation between areal and volumetric estimates of production. The variability is caused by differences in transparency and morphometry between lakes. As long as transparency remains constant, data from any lake plot on a straight line (some of which are apparent in Fig. 5) since the ratio between areal and volumetric rates of production is the mean depth of the euphotic zone. Lakes obviously differ in this value and to eliminate potential errors in the future use of published data, results should be reported in both units since both are required in different applications.

TABLE 3. The ratio of annual production estimates made with actual surface irradiances to those made with simulated cloudless irradiance. "*%Io*" is the value of annual irradiance expressed as a percent of the cloudless annual totals.

Lake \ Year	73	74	75	76
239	.75	.84	.87	.82
226SW	.77	.85	.85	.84
226N	.78	.86	.89	.85
227	.79	.86	.88	.84
302S	.77	.84	.89	.86
302N	.78	.83	.87	.85
304	.79	.87	.91	.86
261	.77	.85	.88	.84
$\bar{x}$	.78	.85	.87	.85
%Io	59	68	71	74

TABLE 4. Coefficients of variation (SD/mean) of annual areal production (corrected for morphometry).

Lake	*n*	Euphotic zone	Epilimnion
Control			
Stratified			
224	3	.098	.229
223	2	.401	.153
239	4	.082	.071
240	2	.264	.199
383	2	.178	.342
Unstratified			
114	2	.147	.147
		$\bar{x}$ = .195	.166
Experimental			
Stratified			
226SW	4	.151	.265
226NE	4	.159	.179
227[a]	4	.569	.628
302S	4	.261	.268
302N	4	.172	.163
304	4	.204	.263
261	4	.224	.215
230	2	.256	.117
Unstratified			
303	2	.265	.265
		$\bar{x}$ = .212	.217

[a]Not used in the calculation of the mean.

Discussion

The procedure used to calculate total primary production from raw data has received little consideration. Data have almost universally been reported as g C/m^2 and refer to integral production of a column of water located at the center of the lake. In general, multiplication of this figure by the surface area of the lake does not give the true total carbon fixation, however, because peaks of production that occur near the bottom of the euphotic zone are associated with less water than those at shallower depths while the standard calculation procedure weights peaks at all depths equally. With the exception of Kerekes (1975), published data either contain this systematic error, or else it is not clearly stated how morphometric corrections were applied. Figure 2 suggests that epilimnion results reported as average production per unit volume are

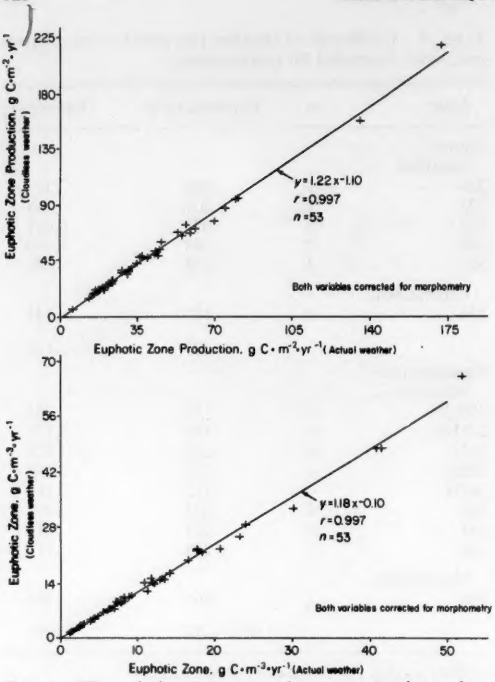
Euphotic Zone Production, g C·m⁻²·yr⁻¹ (Cloudless weather)
y = 1.22x - 1.10
r = 0.997
n = 53
Both variables corrected for morphometry
Euphotic Zone Production, g C·m⁻²·yr⁻¹ (Actual weather)
Euphotic Zone, g C·m⁻³·yr⁻¹ (Cloudless weather)
y = 1.18x - 0.10
r = 0.997
n = 53
Both variables corrected for morphometry
Euphotic Zone, g C·m⁻³·yr⁻¹ (Actual weather)

FIG. 4. The relation between primary production calculated with actual weather (x axis) and calculated with simulated cloudless weather (y axis). Separate graphs are shown for areal (above) and volumetric (below) units.

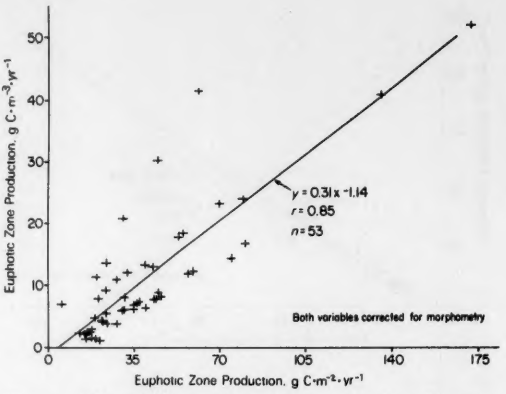
Euphotic Zone Production, g C·m⁻³·yr⁻¹
y = 0.31x - 1.14
r = 0.85
n = 53
Both variables corrected for morphometry
Euphotic Zone Production, g C·m⁻²·yr⁻¹

FIG. 5. The relation between areal and volumetric rates of primary production in ELA lakes.

negligibly affected by this correction. However, if data are reported in areal units or if a stratified water column is considered, then up to 20% calculation error could be present.

Probably the error will be smaller in larger lakes. Small lakes (only one of the 19 lakes in this study was larger than 56 ha) are those in which the calculation error will be maximal, because the area/volume ratio is very large. The magnitude of this scale effect can be gauged by the L228 result — the only reasonably large lake considered here — where the calculation error was only 6%.

Another important effect of small surface area is that it leads to shallow thermoclines. Together with the high transparency of ELA lakes, this allows the development of unusually large hypolimnion algal blooms (Fee 1976). These populations exhibit very high ^{14}C uptake per unit volume of water but are associated with little total volume in situ. Together with a systematic technical error (light quality, which alone caused production estimates to be two to five times higher [Fee 1978b]), the uncorrected method of reporting results greatly exaggerated the contribution of these hypolimnion blooms to whole lake primary production. This is a substantive correction of the conclusion of Fee (1976) regarding these populations and serves as an example of the confusion that this type of calculation error can introduce.

Obviously, in larger lakes horizontal inhomogeneities of biomass or transparency will be greater sources of error than the calculation error and existing data on such waterbodies do not need correction. This is not to say that the error can be ignored, however, since convincing experimental work is necessarily restricted to small lakes where the effect is potentially appreciable. In these lakes the correction for morphometry is critical.

THE PRIMARY PRODUCTION MODEL

Finally, I want to use the results to derive some assessment of the relative importance of the phenomena that are included in the numerical photosynthesis model (Fee 1977). The incubator based technique has become increasingly complex with time and to apply the full method appears to be a formidable task. This is unfortunate since this method has much greater precision and accuracy than in situ ones (see below).

To simplify the discussion, the variables have been put into three groups:

Primary variables
- Transparency
- Incubator production vs. light curves
- Morphometry

Secondary variables
- Solar insolation
- Incubator irradiance correction

Tertiary variables
- In vivo chlorophyll profiles
- Diurnal variation of production vs. light curves

The primary variables form the heart of the model and direct measurements are necessary for accurate

results with the method. Published relationships between chlorophyll and quantitative features of production vs. light curves are highly inconsistent. For example, Hecky et al. (1978) showed that the assimilation number, which is the ratio of carbon uptake at saturating light to chlorophyll concentration, varied from 0.2 to 20 in Lake Tanganyika. Further, Fee (1978b) showed that small errors in the slope of the photosynthesis vs. light curves cause two to five times overestimation of production at low irradiances. No general procedure exists which will allow accurate prediction of these quantitatively important features of photosynthesis vs. light curves from chlorophyll concentrations alone. Shearer (1976c) has described the construction and operation of an incubator that has been used to make these measurements. Transparency measurements are easily made directly. The importance of the other primary variable — morphometry — has already been discussed above.

The secondary variables are those that are of crucial importance in certain contexts but are such that they may be neglected in others. Gathering and reducing surface irradiance data is a tedious part of the method and based on the results in Table 4 it would seem justified to eliminate this step for ELA annual production estimates. This does not mean that actual variations of surface irradiance are unimportant to production: daily production values varied widely as a result of cloudiness. Even the annual figures show the dependency of these variables. The 9% increase in annual irradiance from 59% in 1973 to 68% in 1974 resulted in a 7% production increase. Since annual production must be 100% when annual irradiance is 100%, a further 22% increment of irradiance must result in a 15% increment of production. The curve relating these two variables is therefore hyperbolic. Above about 65% of possible irradiance this curve is quite flat and over a broad range of annual irradiances only about a 5% error in the estimate of annual production will result by assuming it to be constant instead of measuring it. Results from Southern Indian Lake (R. Hecky personal communication) in northern Manitoba and from Lake Tanganyika (Hecky et al. 1978) in equatorial Africa have also shown annual integral production to be about 80% of the value predicted with the cloudless model. These three regions are all relatively sunny, however. In much more cloudy areas, where actual insolation is a much lower percentage of the cloudless figure (say less than 50%), the ratio could alter significantly. Further, to obtain an accurate formula for cloudless weather according to the method of Fee (1977), at least one season of insolation records is needed. I suggest, therefore, that investigators experimentally confirm the ELA result for at least one season before discontinuing insolation measurements.

The other secondary variable is the incubator irradiance correction for light quality (Fee 1978b). It is necessary to consider this only in lakes where subepilimnion production is being monitored. In these lakes it must be considered to be an essential step in the method since errors of two to five times can result from neglecting it. Although strictly necessary only in lakes where subepilimnion production is important, I recommend that this procedure be adopted in all studies since it forces incubator results to generate actually monitored in situ results.

Tertiary variables are of minor quantitative importance in most waterbodies. Fee (1975) showed that except in the most extreme cases, ignoring diurnal variation of photosynthesis vs. light curves caused less than a 10% overestimate of production. Further, results from Southern Indian Lake (R. Hecky personal communication) and Lake Tanganyika (Hecky et al. 1978), as well as the ELA results, showed no single pattern of variation and probably as much error is introduced by forcing a modellable pattern on these results as is removed by including this factor. This conclusion, of course, does not apply to some highly eutrophic soft water lakes that have high pH and low dissolved inorganic carbon (DIC) concentrations due to algal growth. The DIC in such lakes can undergo extreme diurnal fluctuations which severely limit photosynthesis for all but a few hours after sunrise (Schindler and Fee 1973). It is fortunate that such conditions are rare since they require entirely different procedures that account for atmospheric invasion of DIC to accurately estimate production.

It has been shown in this paper that the exact details of the vertical distribution of phytoplankton biomass measured as chlorophyll with an in vivo fluorometer system are not needed to obtain accurate estimates of production. Epilimnion waters are normally well mixed (Fee 1978a) and, as demonstrated by Fee (1976), integrated sampling alone provides accurate estimates of production in subepilimnion chlorophyll peaks by trading off errors. The advantage of using in vivo chlorophyll profiles in the production model is that it gives vertical details of production which may be needed for some purposes. With integrated samples the areal or average production per unit volume of the lake will be accurately estimated but the calculated rates at any specific depth will be unreliable.

Cautious extrapolation of the ELA results to other waterbodies could significantly simplify the application of the incubator method. Investigators are warned to carefully verify that such simplified procedures are justified, however, or unreliable answers could result. Another important caveat is that the recommended simplifications are valid only for estimating annual production. For example, if the purpose of the study is to understand a system over a brief period then significant errors would arise unless the detailed variations of solar insolation were included.

In its most general form the model is applicable to any situation. There is nothing exotic about the method; it is simply the application of modern data processing techniques to extract the maximum amount of information from experimental data. Eight consecutive years

of results from L239 — the first four with the standard in situ technique (Reid et al. 1975b) and the last four with the incubator method — demonstrate the power of the incubator-based procedure. The in situ method gave values ranging from 48 to 110 g $C \cdot m^{-2} \cdot yr^{-1}$ while the incubator method gave values of 18–21 g $C \cdot m^{-2} \cdot yr^{-1}$. During these 8 yr the lake has been remarkably constant in transparency, chemistry and algal biomass parameters (ELA unpublished data). The reduction of the variance of phytoplankton production estimates from ±100% to ±10% sets new standards for work in this field. Its widespread use would contribute significantly to advancement of our understanding of the ecology of lakes.

Acknowledgments

John Shearer and Doug DeClercq did most of the field work and all of the laboratory experiments. Bob Hecky, Kaz Patalas, and Dave Schindler aided enormously in organizing my thoughts and focusing my writing.

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Relationships Between Dipteran Emergence and Phytoplankton Production in the Experimental Lakes Area, Northwestern Ontario

I. J. DAVIES

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

DAVIES, I. J. 1980. Relationships between dipteran emergence and phytoplankton production in the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 37: 523–533.

Dipteran emergence was monitored at the Experimental Lakes Area (ELA) between 1973 and 1977 in seven lakes of different trophic status. The data were used to develop a number of equations which related the quantity and spatial distribution of average annual emergence to lake productivity. These models explained >94% of the variation in mean emergent biomass among lakes or >76% of the variation in numbers of emergent Diptera in terms of phytoplankton production or phosphorus loading. On average, ELA lakes produced 40.8 dipteran adults (9 mg dry weight) per gram carbon fixed by phytoplankton. A single equation for all lakes predicts the surface distribution of emergent biomass, relative to lake depth at any location, from vertical profiles of phytoplankton production. The mean size of dipteran adults was related to lake depth at the point of emergence and average phytoplankton production. An empirical model which used data on the vertical profile, and lake average, of phytoplankton production was developed to predict the number of Diptera emerging from each depth. The maximum depth of emergence was related to depth of the euphotic zone and average phytoplankton production in each lake. Initial tests suggested that the models may provide useful predictions of dipteran emergence for a wider spectrum of lakes.

Key words: aquatic insects, Diptera, Chironomidae, eutrophication, primary production

DAVIES, I. J. 1980. Relationships between dipteran emergence and phytoplankton production in the Experimental Lakes Area, northwestern Ontario. Can. J. Fish. Aquat. Sci. 37: 523–533.

L'émergence des diptères a été suivie de façon continue dans la région des lacs expérimentaux (ELA) de 1973 à 1977 dans sept lacs de conditions trophiques différentes. On s'est servi des données pour construire plusieurs équations reliant quantité et distribution spatiale de l'émergence annuelle à la productivité du lac. Ces modèles expliquent plus de 94% de la variation de la biomasse émergente moyenne parmi les lacs ou plus de 76% de la variation du nombre de Diptera émergents, en termes de production phytoplanctonique ou de charge de phosphore. En moyenne, les lacs de la ELA produisent 40,8 diptères adultes (9 mg en poids sec) par gC fixé par le phytoplancton. Une seule équation applicable à tous les lacs permet de prédire la distribution superficielle de la biomasse émergente, par rapport à la profondeur des lacs à tout endroit, d'après les profils verticaux de production phytoplanctonique. Nous établissons une corrélation entre la taille moyenne des diptères adultes et la profondeur du lac au point d'émergence, ainsi que la production phytoplanctonique moyenne. Nous avons élaboré un modèle empirique faisant appel aux données sur le profil vertical et la moyenne pour le lac de la production phytoplanctonique, qui permet de prédire le nombre de Diptera émergeant de chaque profondeur. Nous établissons la relation entre la profondeur de l'émergence et la profondeur de la zone euphotique, ainsi que la production phytoplanctonique dans chaque lac. Les essais initiaux donnent à croire que les modèles peuvent fournir des prédictions utiles quant à l'émergence des diptères dans une grande variété de lacs.

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CURRENT interest in the holistic view of limnology outlined by Rigler (1975) has led to the reexamination and development of a number of empirical models. Early relationships stressed the importance of using lake morphometry (Rawson 1955), edaphic factors (Northcote and Larkin 1956), or a combination of these elements (Ryder 1965) to predict plankton standing crops or fish yields. Recently more emphasis has been placed on dynamic processes such as nutrient loading (Vollenweider 1969; Patalas 1972; Dillon and Rigler 1974, 1975; Schindler et al. 1978) or the relationship between primary production and fish yield (Melack 1976). Although it is generally recognized that production of benthic invertebrates is some function of lake trophic status (Johnson 1974), relationships which predict standing crop or production of benthos are based wholly on morphometric (Rawson 1955) or

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TABLE 1. ELA lakes used in this study. Chemical additions (C as sucrose, N as $NaNO_3$, and P as H_3PO_4) were made weekly to the epilimnion of each treated lake during the stratified period. P loading values ($Ip\bar{V}o^{-1}$) are for the emergence study period excluding 1977. Averages of annual phytoplankton production ($\bar{P}\bar{P}$) are for the emergence study period.

Lake	Area ($\times 10^4$ m^2)	Maximum depth (m)	Chemical additions (g·m^{-2}·yr^{-1}) Years	C	N	P	H_2SO_4	$Ip\bar{V}o^{-1}$ (mg·m^{-3})	$\bar{P}\bar{P}$ (g C·m^{-2}·yr^{-1})	Emergence study period	Comments
223	27.27	14.4	1976–77	0	0	0	Weekly	30.0	24.9	1975–77	Acid addition ≈ 18 × background loading rate.
224	25.92	27.4	nil	0	0	0	0	NA	21.0	1976–77	
226 NE	8.32	14.7	1973–77	3.46	1.81	0.34	0	190.8	76.2	1974–77	L226: a double basin lake divided by a plastic curtain.
226 SW	7.77	11.6	1973–77	3.69	1.93	0	0	28.2	47.3	1973–77	
227	5.00	10.0	1969–74	0	7.0	0.5	0	324.6	116.7	1973–75	No dipteran biomass estimates for 1975.
			1974–77	0	2.25	0.46	0				
302 N	12.8	13.8	1972–76	3.73	2.79	0.54	0	278.6	40.2	1973–74	L302: a double basin lake divided by a shallow narrows. No biomass estimates for either basin. North basin: nutrients added to hypolimnion only, P loading not used (see text).
302 S	10.9	10.6	nil	0	0	0	0	29.4	35.6	1973–74	

morphoedaphic (Johnson 1974) factors rather than nutrient loading or primary production. This study examines dipteran emergence and its relationship to phytoplankton production and phosphorus loading in several lakes of different trophic status at the Experimental Lakes Area (ELA).

Rawson (1955) described a hyperbolic relationship between average standing crop of benthic organisms and the mean depth of several large Canadian lakes. These lakes had standing crops of net plankton, and fish yields which were similarly related to mean depth implying that the density of macroinvertebrates in each system was directly related to lake productivity. Although several other authors failed to verify Rawson's model, Johnson (1974) argued that these anomalies resulted from the use of standing crop data rather than measurements of production, particularly in comparisons where turnover ratios (production/biomass) of the benthos differed greatly between lakes. As an alternative he suggested a logarithmic model relating production of benthic invertebrates to Ryder's morphoedaphic index (total dissolved solids/mean depth, Ryder [1965]), but cautioned that regional factors such as climate could play an important modifying role.

Variance introduced by such regional factors as latitude, lake size, and lake type may have been the principal reason why Brylinski and Mann (1973) failed to show a relationship between primary production and either carnivorous or herbivorous benthic production in their preliminary synthesis of worldwide IBP information.

In the absence of regional differences, increased standing stocks of benthos (particularly chironomids) developed in response to artificially induced eutrophication in a lake (Smith 1969) and in a series of experimental ponds (Hall et al. 1970). Dermott et al. (1977) reported that chironomid production in the eutrophic South Basin of Lake Memphremagog exceeded that of the more oligotrophic North Basin as did estimates of phytoplankton and fish production.

The work of Speir and Anderson (1974) on Simulidae suggested that emergence may be a constant fraction of larval production. Welch (1973) showed close agreement between the average number of chironomids emerging from Char Lake and that predicted by the C:N ratio of sediments according to the relationship of Laville (1972, cited in Welch 1973). He suggested that Laville's relationship actually reflects a response of emergence to changes in primary production since the C:N ratio in sediment increases as lakes become more eutrophic. To illustrate this point, Welch calculated that emergence was a relatively constant percentage of primary production (0.1–0.8%) in three quite different systems: an arctic lake, two Oregon sewage lagoons, and a Georgia farm pond.

Because the benthic fauna of the ELA lakes is dominated by Diptera, particularly chironomids, with *Chaoborus* playing a numerically minor role, an emergence sampling program was chosen as the most expedient approach to obtaining an index of dipteran production from each lake for comparison with estimates of primary production.

Materials and Methods

The geomorphology of the Experimental Lakes Area (ELA) has been described by Brunskill and Schindler (1971). Summaries of the whole-lake experiments are given by Schindler and Fee (1974) and by Schindler et al. (1978). The emergence study period, basic morphometric data and experimental treatment for each lake are outlined in Table 1.

Submerged concial emergence traps (basal area 0.1 m^2), made from clear cellulose acetate butyrate sheet (Hamilton 1965), were chosen as the basic sampling unit for this study. Trap collections, unlike sediment samples, required little processing, so several lakes could be monitored for a number of years, throughout each ice-free season. Although less rigorous than a detailed study of secondary production based on larval material from sediment sampling, emergence collections do represent the accrued effects of turnover time, production, and mortality and as such were preferable to measurements of standing crop.

Traps were suspended 1 m below the lake surface from

a float held in position by two laterally placed anchors. This method of anchoring reduced the vertical movement of floats exposed to wave action and ensured that the lake bottom beneath each trap was not disturbed. Traps, with screened (240 μm Nitex® cloth) vents in the cone, were placed directly on the substrate at station depths <1 m and removed at the end of each sampling period. Water circulation through the vents prevented the development of anoxia within these traps.

An areally weighted sampling scheme was chosen to ensure an even distribution of sampling effort. Depth intervals which contained approximately equal areas of lake bottom were calculated for each lake. Sampling stations were placed at the average depth of each interval along transects (usually two or three transects per lake). Typically, this process resulted in shallow stations spaced at 1 m depth increments with deeper ones separated by 2–3 m of lake depth. Traps were cleaned and set for one 24-h period each week (during 1973 traps in both basins of Lake 302 were set twice weekly).

Adult Diptera from the traps were separated according to sex, and counted. Total body length was recorded in one of three categories: $\leq$3.90 mm, 3.91–7.79 mm, $\geq$7.80 mm. Pupal exuviae were measured (total length) and counted. Specimens were preserved either in 70% ethanol or in 3.7% aqueous formaldehyde. Those preserved in ethanol were saved for taxonomic work. Insects preserved with formalin were dried at 60°C for 24 h and weighed to provide biomass estimates.

Annual emergence was calculated by integrating data from the weekly trap collections over the ice-free period in each year. Seasonally integrated emergence from each lake depth, as biomass or numbers of Diptera per unit area, was used to describe the depth distribution of emergence. All other relationships were based on integrated emergence corrected for lake morphometry and expressed per unit of lake surface area. Planktonic primary production was measured by the ^{14}C technique using a light incubator (Fee 1973, 1978; Schindler et al. 1972). Seasonally integrated totals of primary production per unit volume ($g\,C \cdot m^{-3} \cdot yr^{-1}$) or production per unit area corrected for morphometry ($g\,C \cdot m^{-2} \cdot yr^{-1}$) were obtained from Fee (1980, table 2, col. 9) and from Fee (unpublished data). The mean depth of 1% of surface light for each lake was evaluated from data presented by Reid et al. (1975), Shearer (1976), and Shearer and DeClercq (1976, 1977, 1978). Annual phosphorus loading rates for each lake were supplied by Schindler (personal communication).

Relationships outlined in this paper were calculated by linear regression analysis (Snedecor and Cochran 1967). All correlation coefficients were significant at the $P \leq 0.05$ level. In a preliminary analysis relationships were calculated by using data from individual years of the study. A second analysis was performed on the means since dipteran emergence was generally less variable than phytoplankton production from year to year, particularly in the more eutrophic lakes. Means were weighted according to the number of years' data that each represented; 95% confidence bands were calculated by using an appropriately reduced number of degrees of freedom (df). Except where noted, analysis of means forms the basis of the relationships outlined in this paper.

Details of the study-methods and data are currently in preparation for publication as data reports (Canadian Data Report of Fisheries and Aquatic Sciences).

Definition of Symbols

$\bar{B}$	Lake-average dry biomass of emergent Diptera, corrected for morphometry ($g \cdot m^{-2} \cdot yr^{-1}$).
B_{zi}	Average integral biomass of Diptera emerging per unit area from the ith depth interval ($g \cdot m^{-2} \cdot yr^{-1}$).
B_{95}	The depth above which 95% of the lake-total biomass of Diptera emerges (m).
$\bar{E}$	Lake-average number of emergent Diptera, corrected for morphometry (Number $\cdot m^{-2} \cdot yr^{-1}$).
E_{zi}	Average integral number of Diptera emerging per unit area from the ith depth interval (Number $\cdot m^{-2} \cdot yr^{-1}$).
E_{95}	The depth above which 95% of the lake-total number of Diptera emerge (m).
$I_p V_o^{-1}$	Annual total of phosphorus received by a lake divided by the volume of the lake outflow: equivalent to [P] in the inflow corrected for precipitation and evaporation ($mg \cdot m^{-3}$) (see Schindler et al. 1978).
$\bar{N}$	Average number of Diptera emerging per g C of PP_{zi}. Units of Number $m \cdot g\,C^{-1}$ result when estimates of E_{zi} (Number $\cdot m^{-2} \cdot yr^{-1}$) are divided by PP_{zi} ($g\,C \cdot m^{-3} \cdot yr^{-1}$) for each interval of depth.
$\bar{PP}$	Lake-average phytoplankton production, corrected for morphometry ($g\,C \cdot m^{-2} \cdot yr^{-1}$).
PP_{zi}	Average integral phytoplankton production per unit volume in the ith depth interval ($g\,C \cdot m^{-3} \cdot yr^{-1}$).
$\bar{W}$	Mean dry weight of an individual dipteran adult (mg).
$\bar{Z}_c$	Mean depth of 1% of surface irradiance during the ice-free season (m).

Results

Although dipteran emergence from a given depth was highly variable when measured at different locations around each lake on any single collection date, annual totals of emergence from the same stations were approximately constant. Seasonal integrals of emergence were therefore chosen as the most appropriate form of the data for further analysis. Differences in annual emergence between lakes were viewed in two ways: (1) on a whole-lake basis, considering the average biomass and number of adults, and (2) within each lake by examining distribution patterns of emergence.

Whole-Lake Relationships

Overall, lakes with high annual phytoplankton production yielded proportionately high annual emergence (Fig. 1). When data for individual years were used to calculate the relationship between lake-average emergent biomass ($\bar{B}$) and lake-average phytoplankton production ($\bar{PP}$) a relatively low coefficient of determination ($r^2 = 0.541$) was obtained (Fig. 1a). Although

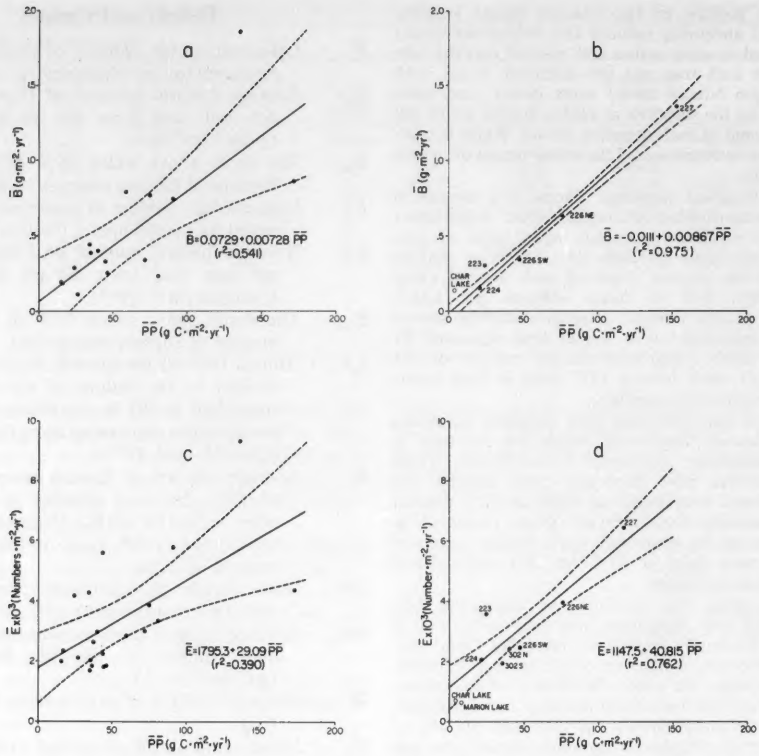

FIG. 1. Relationship of annual dipteran emergence, as lake-average biomass ($\bar{B}$) or numbers ($\bar{E}$), to average annual phytoplankton production ($\bar{P}\bar{P}$) in the study lakes. Plots of the data are shown for individual years (a and c) and as averages over the study period for each lake (b and d). Data for Char and Marion lakes (open circles) were not included in the calculations. Dashed lines show the 95% confidence limits of each regression line.

emergence was generally greater than expected in the year following a year of high phytoplankton production, no improvement in r^2 resulted if values of $\bar{B}$ were paired with estimates of $\bar{P}\bar{P}$ from the previous year. The relationship was considerably improved (Fig. 1b) when data for individual years were replaced by means calculated over the total study period for each lake (Table 1). Much of the variation in the initial relationship may have been due to yearly fluctuations in primary production coupled with the fact that a portion of dipteran production overwinters each year as larvae, then completes its development and emerges in the following year.

A similar relationship (Fig. 1c and d) existed between $\bar{P}\bar{P}$ and the lake-average number of emergent Diptera ($\bar{E}$). As in the previous case, the coefficient of determination was greatly improved when data for individual years (Fig. 1c) were replaced by long-term means (Fig. 1d).

DEPTH DISTRIBUTION OF EMERGENCE

A second important feature of emergence was its distribution within each lake. In eutrophic systems (e.g. Lakes 226 NE and 227) emergence per unit area was maximum in the shallows and declined sharply towards deeper portions of the lake (Fig. 2), while in oligotrophic lakes (e.g. Lakes 223 and 224) the decline of emergence with depth was more gradual. The depth distribution of emergent biomass (as B_{zi}) followed the vertical profile of phytoplankton production (as PP_{zi}) in the same way for all lakes (Fig. 2) suggesting that B_{zi} was some constant fraction of PP_{zi} over a wide range of lake depth and trophic status.

Data from Fig. 2 were grouped into broader intervals of depth to reduce the effects of fluctuations in lake levels, seasonal variation in the depth profile of phytoplankton production and in some cases, a lack of replicate sampling stations. The original number of

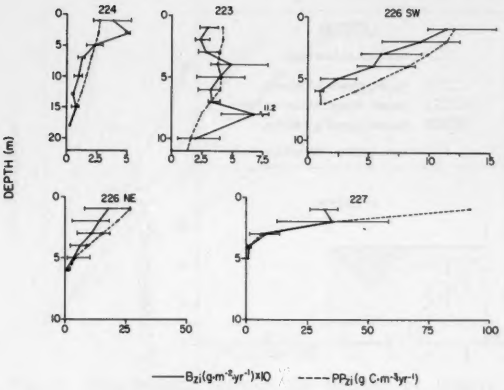

FIG. 2. The depth distribution of mean-annual phytoplankton production in the water column (PP_{zi}, broken line) over i depth intervals in each of five ELA lakes and the mean-annual biomass of dipteran emergence (B_{zi}, solid line) from the same depth intervals. Bars indicate the range of values for B_{zi}. Note the following scale differences: (1) PP_{zi} is 10× B_{zi}, (2) the depth scale for Lake 224 is reduced by 2×, (3) production–biomass scales for Lakes 226 NE and 227 are reduced by 5×.

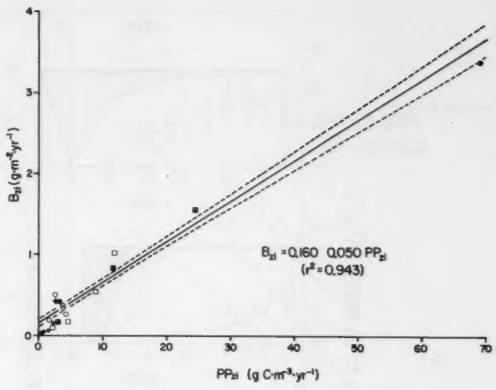

FIG. 3. Biomass of dipteran emergence (B_{zi}) and its relationship to phytoplankton production (PP_{zi}), plotted for several depth intervals in each of the study lakes. Data are expressed as mean seasonal-integrals of emergence per unit area or production per unit volume, not corrected for morphometry. Symbols: Lake 223 (○), Lake 224 (+), Lake 226 SW (□), Lake 226 NE (■), Lake 227 (●). Dashed lines show the 95% confidence limits of the regression line.

intervals was halved by averaging emergence from stations at two adjacent lake depths. A plot of the regrouped data for the broader intervals of depth showed that a single relationship existed between B_{zi} and PP_{zi} for all lakes and depths (Fig. 3). To check the effect of the uneven distribution of points on the regression, data were logarithmically transformed and the least-squares fit recalculated. Because transformation did not substantially alter or improve the result, the relationship was retained in its linear form.

Although the ratio of B_{zi} to PP_{zi} was constant in all of the study lakes, the number of dipteran adults per unit of emergent biomass differed from lake to lake according to its trophic status. The pertinent variable was adult size. Small Diptera made up the majority of emergence in oligotrophic lakes (Fig. 4, Lakes 223 and 224) while larger individuals were more common in euthrophic lakes (e.g. Lakes 226 NE and 227). Size was also a function of depth (Fig. 4). Emergence from the shallows was usually dominated by small individuals but for each lake the percentage of larger individuals in the catch increased steadily with depth.

Because of the variation in adult size, plots of the number of Diptera emerging per unit area from each depth interval (E_{zi}) against PP_{zi} yielded a different relationship for each lake (Fig. 5). In order to predict the form of a relationship between E_{zi} and PP_{zi} for any lake, the secondary influences of lake productivity and station depth on the average size of adult insects must be considered. Although it may have been possible to derive an equation for predicting the average weight of dipteran adults, then use these weights to estimate E_{zi} from B_{zi}, the simplest solution was to empirically determine the combined influence of lake productivity and depth on E_{zi}.

Regression lines fitted to the data (Fig. 5) provided reasonable first approximations of the relationship between E_{zi} and PP_{zi} for each lake, except at low values of PP_{zi}. The equation of each line may be written in the general form:

$$(1)\quad E_{zi} = \bar{N} \cdot PP_{zi}$$

where $\bar{N}$ represents the mean number of Diptera emerging per g C of phytoplankton production. The characteristic slope ($\bar{N}$) is the net result of interactions between the biomass of emergence, average adult size and lake depth. Values of $\bar{N}$ were maximum for oligotrophic lakes (Fig. 5) and declined with increases in overall lake productivity towards a minimum for Lake 227, the most eutrophic of the series. Figure 6 shows that $\bar{N}$ is an inverse function of $\bar{P}\bar{P}$. If $\bar{P}\bar{P}$ is known, values of $\bar{N}$ (derived from the equation in Fig. 6) can be substituted into Equation 1 and approximate solutions for E_{zi} can be calculated for each value of PP_{zi}.

OTHER RELATIONSHIPS

Because of the strong relationship between phosphorus loading (as $I_pV_o^{-1}$) and phytoplankton production in ELA lakes (Schindler et al. 1978), estimates of lake-average emergence (as $\bar{B}$ or $\bar{E}$) may be predicted directly from values of $I_pV_o^{-1}$:

$$(2)\quad \bar{B} = 0.182 + 0.00315\,(I_pV_o^{-1}) \qquad (r^2 = 0.894, df = 4)$$

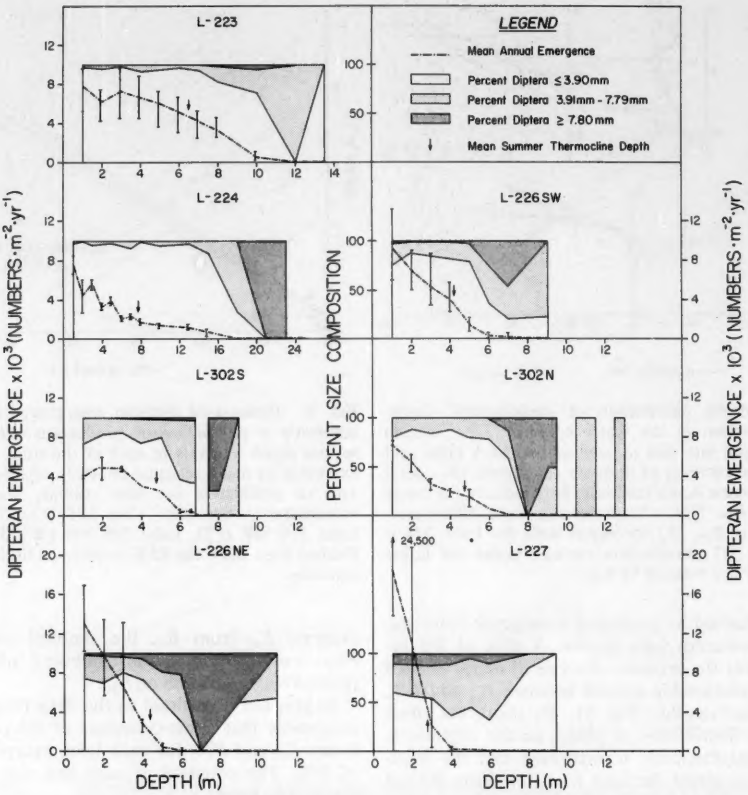

FIG. 4. The depth distribution of size and numbers of emergent Diptera in the study lakes. Data are means of seasonally integrated emergence from each depth, not corrected for lake morphometry. Vertical bars indicate the range of emergence values at each depth.

(3) $\bar{E} = 1957.8 + 12.23\,(I_p V_o^{-1})$ $(r^2 = 0.809, \mathrm{df} = 7)$

In the calculation of Equation 3 the value of $I_p V_o^{-1}$ for Lake 302 N was assumed to be equal to that of Lake 302 S, as phosphorus added to the hypolimnion of the North Basin had little effect on lake average phytoplankton production (Schindler et al. 1980).

It may also be desirable to make limited predictions about the maximum depth of emergence when data on phytoplankton production or its vertical distribution are not available. The depth of the euphotic zone in most lakes is inversely related to trophic state, except in cases where silt loads or highly colored water act to extinguish light. Commonly the mean depth of 1% of surface light ($\bar{Z}_c$) is thought to mark the lower limit of photosynthesis. Since emergence and primary production were closely related (Figs. 1b, 1d, 3 and 5), the depth above which 95% of the lake-total emergence occurs, as numbers (E_{95}) or as biomass (B_{95}), was related to the depth of the euphotic zone as follows:

(4) $B_{95} = 1.154 + 0.972\bar{Z}_c$ $(r^2 = 0.848, \mathrm{df} = 9)$

(5) $E_{95} = -0.106 + 0.819\bar{Z}_c$ $(r^2 = 0.976, \mathrm{df} = 12)$

where $\bar{Z}_c$ is the average depth of 1% of surface light for the ice-free season.

Since $\bar{Z}_c$ is also defined as the lower limit of primary production it follows that B_{95} and E_{95} are related to $\bar{P}\bar{P}$.

(6) $B_{95} = 239.56(\bar{P}\bar{P})^{-0.884}$ $(r^2 = 0.980, \mathrm{df} = 9)$

(7) $E_{95} = 120.66(\bar{P}\bar{P})^{-0.808}$ $(r^2 = 0.923, \mathrm{df} = 12)$

The exponential form of Equations 6 and 7 comes from the fact that both primary production and dipteran emergence are related to each other through the light extinction curve.

Phytoplankton production is usually reported in units of $g\,C \cdot m^{-2} \cdot yr^{-1}$; details of its vertical distribution are often omitted. By using the relationships outlined either in Equations 6 and 7 or in Equations 4 and 5 the

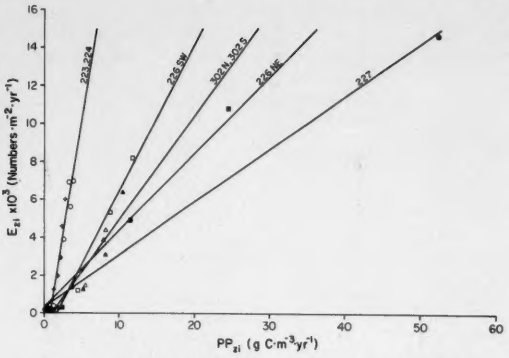

FIG. 5. Relationships between the number of Diptera emerging from each depth interval (E_{zi}) and phytoplankton production in the same intervals (PP_{zi}) for seven ELA lakes. Data are expressed as mean seasonal-integrals of emergence per unit area or production per unit volume, not corrected for lake morphometry. Symbols: Lake 223 (○), Lake 224 (+), Lake 226 SW (□), Lake 226 NE (■), Lake 227 (●), Lake 302 S (△), Lake 302 N (▲).

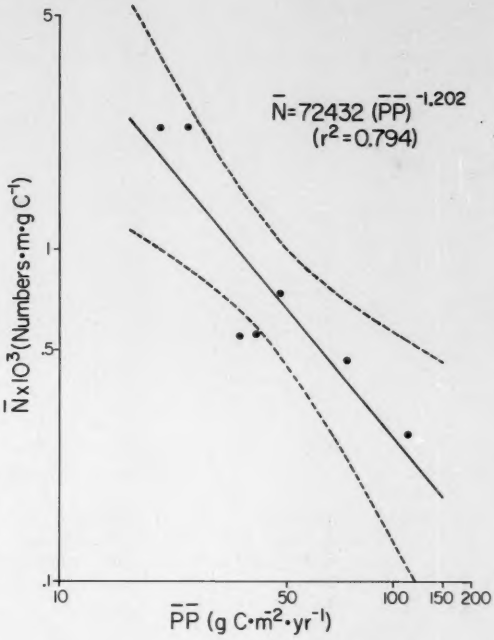

FIG. 6. Number of Diptera ($\bar{N}$) emerging per g C of lake-average phytoplankton production ($\bar{\bar{PP}}$). Dashed lines show the 95% confidence limits of the regression line.

maximum depth of emergence can be calculated. Values of B_{95} and E_{95} may be used in combination with data on lake morphometry to modify estimates of $\bar{B}$ and $\bar{E}$ from the regression equations in Fig. 1b and d or from Equations 2 and 3 to expressions of average B_{zi} and E_{zi} over the zone of emergence (i.e. $zi = Om$ to B_{95} or Om to E_{95}). Although values of B_{zi} and E_{zi} calculated in this way have limited application, they are useful for estimating trap size and the distribution of sampling effort needed to establish an effective emergence sampling program in a previously unstudied lake.

Discussion

Equations outlined in this study offer a number of simple approaches to predicting dipteran emergence from lakes. The choice of phytoplankton production as the central variable in these relationships was determined by the strength of its correlations with emergence and the fact that of all the parameters tested for their ability to predict emergence (phytoplankton production, phosphorous loading, light, temperature, and oxygen) it was the only one related to both lake-average emergence and its distribution with depth. Overall, phytoplankton production explained more than 94% of the variation in the depth distribution of emergent biomass and more than 97% of the variation in the average biomass of emergence among lakes.

The latter relationship may be written as net conversion efficiency by expressing biomass as a percentage of phytoplankton production. Table 2 summarizes literature values of conversion efficiency for dipteran emergence. If 50% of the dry weight of emergence is carbon (Winberg 1971), conversion efficiency for ELA lakes is 0.4%. This falls within the surprisingly low range of literature values from 0.1 to 2.2%.

Speir and Anderson (1974) suggested that emergence represents a fixed proportion of dipteran production. Their data, and additional values calculated from the literature, are shown in Table 2. Assuming an average emergence to production ratio of 0.25 for ELA lakes, annual dipteran production represented 1.7% of phytoplankton production (^{14}C estimate).

Whole-lake relationships (Fig. 1) were tested for their ability to predict emergence for lakes outside the ELA region. With the possible exception of a hypereutrophic sewage treatment lagoon, the data for other lakes (Table 3) showed a positive relationship between emergence and primary production. Reported values of dipteran numbers and biomasses for both Char and Marion lakes fell within the 95% prediction limits of the regression equations in Fig. 1b and d (see Table 3). Even though ELA relationships tended to underestimate biomass and overestimate dipteran numbers for the other systems, it should be noted that phytoplankton production in each of the six examples lay outside the range of values used to derive the models.

TABLE 2. Dipteran emergence as percentages of phytoplankton gross primary production (GPP), and secondary production of the diptera taxon considered. Asterisks mark reported values, others were calculated from data presented in the papers referenced.

Lake	Dipteran taxon	GPP ($m^{-2} \cdot yr^{-1}$)	Emergence as % of GPP	Emergence as % of taxon production	Reference
Char	Chironomidae	4.2 g C	1.7	33.8	Welch (1973, 1976) Welch and Kalff (1974)
Oregon streams	Simulidae	NA	NA	21.7*	Speir and Anderson (1974)
ELA lakes	Diptera	Assuming 50 g C	0.4	NA	This study
Cornell ponds	Chironomidae	NA	NA	7.9*	Hall et al. (1970)
Esrom	Chironomidae	2 440 kcal	1.7	52.5	Jónasson (1972)
Esrom	Diptera	2 440 kcal	2.2	56.9	Jónasson (1972)
Lago Pond	Chironomidae	7 389 kcal	0.8*	20.0	Welch (1967, 1973)
Lago Pond	Diptera	7 389 kcal	1.0	22.3	Welch (1967)
Primary lagoon	Chironomidae	35 000 kcal	0.1	38.4	Kimerle and Anderson (1971)
Secondary lagoon	Chironomidae	27 790 kcal	0.4	40.3	Kimerle and Anderson (1971)

Periphyton production was not included as a variable in any of the relationships as it represented only 1–2% of phytoplankton production in lakes typical of the ELA region (Schindler et al. 1973). Although periphyton may be an important food resource for some dipteran species, its production alone was insufficient to maintain the levels of emergence measured in the ELA lakes (assuming that emergence represents 25% of larval production). This may not be true for Marion and Char lakes where periphyton were major contributors to algal production (Efford 1972; Welch and Kalff 1974). To see if emergence predictions for these two lakes were improved by including periphyton production, total algal production was substituted for $\bar{P}\bar{P}$ and the estimates of $\bar{B}$ and $\bar{E}$ were recalculated (Table 3). Moss production was excluded as it did not represent a food resource for Char Lake chironomids (Davies 1975). Similarly, macrophytes were assumed to be unimportant as food in Marion Lake. Recalculated estimates of emergent biomass for Char Lake agreed well with reported values but, for both lakes, predictions of the number of emergent Diptera moved further away from the observed values. From these few data it was impossible to determine whether phytoplankton production alone could provide a reasonable estimate of dipteran emergence in lakes where total algal production is dominated by periphyton.

The single equation relating B_{zi} and PP_{zi} for all the study lakes (Fig. 3) suggests a possible mechanism for the relationships between emergence and phytoplankton production. Predictions of B_{zi} depend only on PP_{zi} and are not modified by lake trophic status, station depth, temperature, oxygen, or light, implying that food, rather than environmental factors is of primary importance in determining the total biomass of larvae at each depth and ultimately, the pattern of emergence.

TABLE 3. Comparisons between reported values of emergence as biomass or numbers of Diptera and those calculated (±95% confidence limits of the prediction) from estimates of $\bar{P}\bar{P}$ using the regression equations in Fig. 1b and d. Asterisks mark estimates of $\bar{P}\bar{P}$ modified to include epibenthic algal production.

Lake	$\bar{P}\bar{P}$ ($g C \cdot m^{-2} \cdot yr^{-1}$)	Biomass ($g \cdot m^{-2} \cdot yr^{-1}$) Observed	Biomass Predicted	Biomass (±95% CL)	Numbers ($m^{-2} \cdot yr^{-1}$) Observed	Numbers Predicted	Numbers (±95% CL)	Comments	Reference
Char	4.2	0.143	0.025	(±0.147)	690	1 320	(±1 740)	Oxygen to carbon conversion factor for non-moss primary production = 0.355	Welch (1973) Welch and Kalff (1974)
Char	12.9*	0.143	0.101	(±0.145)	690	1 670	(±1 710)		
Marion	8.0	NA	NA		634	1 470	(±1 730)		Hamilton (1965) Efford (1972)
Marion	50.3*	NA	NA		634	3 200	(±1 640)		
Esrom	260	9.40	2.24	(±0.22)	6 370	11 800	(±2 900)	Emergent biomass reported for profundal zone only. Numbers calculated from biomass estimates and pupal dry weights.	Jónasson (1972)
Lago Pond	738.9	14.3	6.40	(±0.58)	34 800	31 300	(±8 000)	Numbers calculated from biomass estimates and mean adult weights.	Welch (1967)
Primary lagoon	3500	6.69	30.3	(±2.9)	3 560	144 000	(±39 000)	Assuming: Mean adult dry wt. = 1.88 mg and 5.25 kcal $\cdot g^{-1}$ of Diptera.	Kimerele and Anderson (1971)
Secondary lagoon	2779	19.0	24.1	(±2.3)	10 100	115 000	(±31 000)		

If algae are used directly, either as phytoplankton filtered from the water column or as periphyton grazed from the substrate, it becomes important to consider the distribution of this food resource within a lake. Because production is a process which is continuous in time, measurements of algal standing crop at each depth may be unrelated to phytoplankton production. It is, therefore, more appropriate to consider the distribution of algae in terms of lake mixing processes.

Quay (1977) reported that horizontal mixing within the thermocline and hypolimnion of two ELA lakes (Lakes 224 and 227) was between 10^3 and 10^5 times greater than vertical mixing. Although mixing rates in the hypolimnion were slower than epilimnetic rates by a factor of 10^2, a point source of tracer (^3H_2O) was mixed uniformly over a horizontal plane in each lake in less than 10 d. For a horizontally mixed, but vertically stable system the depth distribution of the larval food resource should reflect the vertical profile of phytoplankton production.

Within the epilimnion, algal production has two components: a fixed or epibenthic portion and a planktonic portion which appears to be uniformly distributed throughout the mixed layer. The sum of these components yields a net profile of algal availability which declines with depth. The vertical gradient of phytoplankton production may act to reinforce this profile whenever epilimnetic circulation is reduced by periods of calm or secondary temperature-stratification. By assuming that profiles of "available" algae and phytoplankton production are similar the latter variable may be used to predict emergence at each depth.

There is disagreement among authors as to the relative importance of algae, detritus, and bacteria in the diet of larval chironomids (Davies 1975). If, instead of algae, detritus, and its associated bacterial component is assumed to be a major food resource, emergence and the amount of sediment deposited at each lake depth should be related. Although patterns of sedimentation were not measured, the largest net accumulations of sediment occurred in the deeper portions of each lake, not in the shallows, suggesting that if larval growth and abundance are influenced by sediment buildup the effect is largely inhibitory. Further, Bjarnov (1972) and Monk (1976) have shown that cellulose, a major component of detritus is digested poorly, if at all, by most Diptera.

The relationship between B_{zi} and PP_{zi} (Fig. 3) seemed to be unaffected by the presence of *Chaoborus* (a carnivorous dipteran) in some of the lakes. Although this species is restricted to the hypolimnion for much of the year, Borutsky (1939) and Welch (1967) have reported that *Chaoborus* larvae migrate shoreward just prior to pupation. This migration, and relatively low numbers of *Chaoborus*, may explain the lack of emergence at the deepest stations.

Increases in the mean weight of dipteran larvae and adults which developed in response to eutrophication have been noted by several authors (Jónasson 1969; Smith 1969; Hall et al. 1970; Dermott et al. 1977). These increases have been attributed to improved growth conditions for individual species (Hall et al. 1970; Dermott et al. 1977) or to shifts in species composition (Jónasson 1969). For ELA lakes the mean weight of emergent adults ($\bar{W}$), calculated from annual totals of numbers and biomass, was closely related to average phytoplankton production ($\bar{P}\bar{P}$) as follows:

$$(8)\quad \bar{W} = -0.138 + 0.069 \ln \bar{P}\bar{P} \qquad (r^2 = 0.973, \mathrm{df} = 9)$$

Although average adult weight increased as lakes became more eutrophic (Eq. 8), the modifying influence of depth on the distribution of individual weight within a population made it impossible to predict the number of Diptera emerging at each depth (E_{zi}, Fig. 5) from estimates of biomass (B_{zi}, Fig. 3) and $\bar{W}$ (Eq. 8). Instead, an empirical factor ($\bar{N}$, Fig. 6) which incorporated the effects of lake depth and trophic status served this purpose. To explain the observation that large Diptera are frequently associated with the deepest portions of lakes Jónasson (1969) proposed that as oxygen gradients develop in the hypolimnion near the sediment–water interface only large larvae are able to break down the microstratification and irrigate their respiratory surfaces with oxygenated water from above. The work of Sweeney and Vannote (1978) suggested that relatively constant temperatures in the hypolimnion should promote maximum growth in cold-adapted species, while the wide range of temperature in the surface waters was more likely to produce suboptimal growth and smaller size. Other variables such as local conditions of strong winds, currents, or persistant ice cover all affect the success of emergence and may act to increase adult size, while biological factors such as predation may make small body size an advantage.

Although the data were insufficient to determine the actual mechanisms governing the size of individual Diptera or the magnitude and distribution of emergence, relationships developed in this study demonstrate that each of these features is highly predictable for ELA lakes from estimates of annual phytoplankton production and its depth distribution. Average values of emergent biomass or dipteran numbers and the maximum depth of emergence may also be calculated from physical–chemical parameters such as phosphorus loading and light extinction measurements. Comparison of the ELA-based predictions with data from other systems indicated that even though factors such as lake size and climate may play an important modifying role, the basic models should apply to a much wider variety of lakes.

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Comparison of Fin-Ray and Scale Age Determinations for Lake Whitefish (*Coregonus clupeaformis*) and Their Implications for Estimates of Growth and Annual Survival

K. H. MILLS

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

AND R. J. BEAMISH

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

MILLS, K. H., AND R. J. BEAMISH. 1980. Comparison of fin-ray and scale age determinations for lake whitefish (*Coregonus clupeaformis*) and their implications for estimates of growth and annual survival. Can. J. Fish. Aquat. Sci. 37: 534–544.

Fifteen populations of lake whitefish (*Coregonus clupeaformis*) were sampled to compare the fin-ray and scale aging techniques. There was good agreement between aging methods in fast growing populations, but usually fin-ray ages were larger than scale ages in slow-growing populations. The validity of each aging method was tested by comparing fin-ray and scale ages taken from three populations of whitefish when marked and when recaptured at least 1 yr later. When growth was rapid, recaptured fish formed annular marks on both fin-rays and scales. When growth was slower, annular marks were distinguished on fin-ray sections more often than on scales of recaptured fish. When growth was extremely poor, annular marks on scales of recaptured fish were rarely distinguishable, but still recognizable on the majority of fin-ray sections. When fin-ray ages were consistently larger than scale ages for a given population, fin-ray based estimates of annual survival were higher and growth slower than corresponding scale age based estimates. In general, the fin-ray method was more reliable than the scale method of aging lake whitefish.

Key words: lake whitefish, age determination, scales, fin-rays, annual survival, growth

MILLS, K. H., AND R. J. BEAMISH. 1980. Comparison of fin-ray and scale age determinations for lake whitefish (*Coregonus clupeaformis*) and their implications for estimates of growth and annual survival. Can. J. Fish. Aquat. Sci. 37: 534–544.

Dans le but de comparer les rayons de nageoire et les écailles dans la détermination de l'âge, 15 populations de grands corégones (*Coregonus clupeaformis*) ont été échantillonnées. Les deux méthodes s'accordent bien chez les populations à croissance rapide mais, chez les populations à croissance lente, les âges déterminés par les rayons de nageoire sont plus élevés que les âges scalaires. Nous avons vérifié la validité de chaque méthode en comparant l'âge par les rayons de nageoire et les écailles prélevés sur trois populations de grands corégones marqués et recapturés au moins une année plus tard. Quand la croissance est rapide, les poissons recapturés portent des marques annulaires à la fois sur les rayons de nageoire et sur les écailles. Quand la croissance est plus lente, les marques annulaires apparaissent sur les coupes de rayons de nageoire plus souvent que sur les écailles des poissons recapturés. Avec croissance très lente, les marques annulaires sur les écailles des poissons recapturés sont à peine distinctes, mais peuvent encore être reconnaissables sur la majorité des coupes de rayons de nageoire. Quand les âges déterminés par les rayons de nageoire sont uniment supérieurs aux âges scalaires dans une population donnée, les estimations de la survie annuelle fondées sur les rayons de nageoire sont plus élevées et la croissance plus lente qu'avec les estimations correspondantes fondées sur les écailles. En général, les rayons de nageoire sont plus fiables que les écailles pour déterminer l'âge du grand corégone.

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THE purpose of this study was to compare the scale and fin-ray methods of age determination for lake whitefish (*Coregonus clupeaformis*), examine the validity of each method in whole lake mark–recapture experiments, and discuss some consequences of aging errors on estimates of growth and annual survival.

Scales are the traditional structures used for aging lake whitefish and most other freshwater teleosts (Everhart et al. 1975), but tests of the method's validity are conflicting. Van Oosten (1923) and Hogman (1968) held lake whitefish under artificial conditions to determine whether annuli were formed yearly on scales.

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Van Oosten could identify annuli on scales from whitefish held under constant conditions in aquariums, and Hogman found two distinguishable marks formed on scales of fish held in hatchery pools. Neth (1955) compared scale ages of lake whitefish at release with those at recapture 1 yr later in a whole lake study and found most fish formed one annulus, but felt that older, slower growing individuals might be underaged. Sych (1971) compared scale ages from Swedish coregonids with known ages and found frequent underaging in many populations. Crowding of annuli, failure of annulus formation, resorption or difficulties in interpretation of scales are widespread for in vivo populations (see Carlander (1974) for a review).

The scale method has rarely been compared with other methods of aging. Notable exceptions are Aass (1972) who compared otolith and scale ages using the cisco *Coregonus albula*, Beamish and Harvey (1969) who compared fin-ray and scale ages using the white sucker (*Catastomus commersoni*), and Power (1978) who compared scale and otolith ages for lake whitefish. All three studies showed that when differences between aging methods occurred, scale ages were usually lower than corresponding ages determined by the alternative method.

Alternatives to scale aging are available for most species, but generally researchers have resorted to these only when scale aging was very difficult. Deelder and Williamsee (1973) showed that fin-rays were acceptable alternatives to scales for many European species. Cuerrier (1951) showed fin-ray aging was feasible for lake whitefish and this aging method was chosen as an alternative for this study.

Age composition data are necessary for calculating many population parameters, but rarely are errors in ages considered a serious source of bias. The argument frequently used is that errors would affect only a small portion of a sample, generally old individuals which make up only a small part of the population (Le Cren 1974).

Materials and Methods

Lake whitefish were collected from 15 Canadian lakes using gill nets in all lakes. Trap nets were also used in L226 (Lake 226). Seven lakes were located within the Experimental Lakes Area, Ontario. The other eight were located across central and northwestern Canada. Two lakes, L226 and L302, were divided into halves using vinyl walls for whole lake experiments in eutrophication (Schindler and Fee 1974). Lakes were selected to represent wide ranges of surface area, maximum depth, and geographical location (Table 1). Only South Bay, Manitoulin Island, was sampled in Lake Huron. In other lakes larger than 1,000 km² whitefish were gathered from more than one location.

After fish were weighed, fork length recorded, and sex determined where possible, at least three scales and two fin-rays were removed from each fish. Scales were taken from either the first or second row above the lateral line beneath the posterior portion of the dorsal fin (Johnson 1976; Healey 1975; Hogman 1968; and others). Fin-rays were clipped from either a pelvic or pectoral fin as close to the base of the fin as possible. Ages determined from pectoral and pelvic fins from the same fish do not differ significantly (K. H. Mills unpublished data).

TABLE 1. Location, whitefish sample size, and some physical characteristics of the study lakes.

Lake	Location	Surface area (km²)	Maximum depth (m)	Sample size
Bell	46°N,81°W	—	—	36
Clay	50°N,93°W	30	24	111
Dezadeash	61°N,137°W	77	8	213
ELA: 122		<1	13	200
226		<1	15	410
259		1	20	43
302	49°N,93°W	<1	14	76
305		<1	33	37
310		<1	20	78
468		3	25	82
Great Slave	62°N,114°W	27,195	625	62
Huron	45°N,32°W	59,596	229	164
Opeongo	45°N,48°W	59	52	100
Southern Indian	57°N,99°W	2,250	37	415
Winnipeg	52°N,98°W	24,500	37	308

To determine whether whitefish formed one annulus per year on both scales and fins, fish captured in L226 and L302 were marked and returned to their respective capture locations after sampling. Fish with fork lengths greater than 15 cm were tagged with either individually numbered dart or modified spaghetti tags (White and Beamish 1972). A minimum of 1 yr elapsed after release of marked fish before recaptures were sampled again during routine fall trapnetting and gillnetting.

Three additional scales were removed from recaptured fish from the same area as those taken previously, and at least two fin-rays were removed from the unclipped pelvic fin for use in age determinations. Rays were removed from a pectoral fin of fish which had had both pelvic fins clipped previously. Clipped fin-rays did not regenerate on whitefish larger than 22 cm and in smaller fish the regenerated rays were deformed and easily recognizable. Regenerated fin-rays or scales were not used for any age determinations. All age determinations using fin-rays or scales were made without reference to lengths of fish or previously assigned ages.

Scales were read by scale readers in whatever manner each thought appropriate. All readers had extensive prior experience in aging whitefish; two had routinely aged whitefish for over 20 yr using scales. Van Oosten (1923, 1929) established the criteria used for annulus determination: the crowding of circuli, "cutting over" of circuli on the antero-lateral fields, or both (Bell et al. 1977; Christie 1963; Edsall 1960; and others). Six different readers read the same set of scales taken from L310 whitefish. Three collaborated for one set of readings; the others read the scales independently. Sets of scales taken from lakes other than L310 were aged by one or another of the above readers. The same individual read all scales taken from L226 and L302 whitefish for each of the 3 yr of sampling.

Fin-rays were mounted in clear epoxy, dried, and sec-

TABLE 2. Summary of percent agreement between pairs of fin-ray and scale ages for lake whitefish from 15 populations.

Lake	Scale = Fin-ray	Scale = Fin-ray ± 1 yr	Scale > Fin-ray	Fin-ray > Scale
Huron	93	100	2	5
Clay	79	96	7	14
Winnipeg	55	88	19	26
Bell	42	72	19	39
122	30	75	18	52
226	47	60	3	50
302	41	59	1	58
Southern Indian	27	56	9	64
Opeongo	26	40	1	73
Dezadeash	23	48	1	77
Great Slave	23	60	21	56
259	18	47	1	79
468	16	29	0	84
310[a]	15	49	5	79
305	8	11	0	92

[a]The best agreement between fin-ray and scale readers.

tioned with jewelers' saws using 7/0 or 8/0 blades. Individual sections about 0.5 mm thick were brushed clean and mounted on slides for age determinations under a compound microscope. The translucent zone which appeared as a clear ring extending completely around the center of individual rays under transmitted light was considered an annulus. Fin-ray annuli were similar in appearance to those described by Beamish (1973), Beamish and Harvey (1969), and Scidmore (1953) for white sucker. Two individuals read fin-ray sections from L310 fish independently and sets of fin-ray sections from other lakes were read by one or the other of these readers. The same individual read all L226 and L302 fin-ray sections.

We chose growth curves and annual survival estimates to demonstrate some effects of aging disagreements on population parameters. These were calculated for populations where more than 70 whitefish were sampled. Smaller sample sizes resulted in aberrant growth curves and age-frequency distributions unsuited for catch curve analysis. Growth curves were constructed from mean length at age data using each aging method. Survival was estimated by catch curve analysis (Robson and Chapman 1961) using length frequency distributions based on each aging method.

Results

COMPARISONS OF FIN-RAY AND SCALE AGES

Agreement between fin-ray and scale ages was generally poor for most populations (Table 2). The 15 populations could be separated into two groups based on agreement between pairs of corresponding fin-ray and scale ages.

The first group consisted of five populations and was characterized by generally good agreement between aging methods. Either pairs of fin-ray and scale age agreed for most individuals or differences between the ages were rarely greater than 1 yr. Fish collected from Clay Lake, Lake Huron (Fig. 1), Lake Winnipeg, Bell

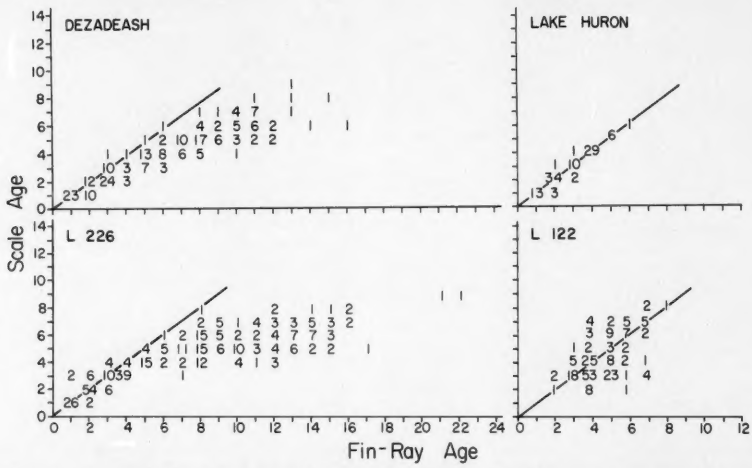

FIG. 1. Comparisons of fin-ray, scale age pairs for lake whitefish from four populations. The Lake Huron and Lake 122 plots are representative of group 1 populations. The Dezadeash Lake and Lake 226 plots are representative of group 2 populations. See text for complete explanation. Numbers represent individual pairs of ages falling in each age category. The diagonal line indicates comparisons where fin-ray ages = scale ages. Comparisons where scale ages > fin-ray ages are above the line; comparisons where fin-ray ages > scale ages are below the line.

Lake, and L122 (Fig. 1) comprised this group. Few individuals from all five populations were aged older than 6 by either method of aging.

The second group was composed of the 10 remaining populations and was characterized by poor agreement between aging methods and many comparisons where fin-ray ages were more than 1 yr greater than scale ages. Many individuals were aged as 8 or older using fin-rays. Few were aged as old as 8 using scale ages.

The poorer the agreement was between methods of aging in the second group, the younger the age-classes which showed consistent differences. In populations where agreement between methods was approximately 35–50%, as in L226 (Fig. 1), fin-ray ages were consistently greater than scale ages, starting at scale ages of 4 or 5. In populations where agreement was 30% or less, like Dezadeash Lake (Fig. 1), consistent differences in aging started at scale ages of 2 or 3. Individuals with greater scale ages could have fin-ray ages two or three times those of the corresponding scale ages.

The L310 population sample exhibited poor agreement between methods and was well suited for an examination of replicability between readers. Agreement between fin-ray readers (61%) was better than agreement between any combination of scale readers (44%). The best agreement between any combination of fin-ray and scale readers was 15% and the worst agreement was 6%. While the fin-ray ages of one reader were slightly higher than those of the second, the differences were much less than those between scale readers. The variation in age assigned a particular fish was sometimes large, as much as 14 yr between a fin-ray and a scale age. See Beamish et al. (1976) for a more detailed discussion of these results.

Fish length was a poor indicator of fish age for L310 whitefish, as was generally true for other populations in this second group. While the smallest individuals were normally younger than the largest ones, large variations in age within 1 cm length groups obscured any clear relationship. More variation within these length groups was apparent using fin-ray ages rather than scale ages in the L310 sample, by as much as 11 yr, but scale ages also varied widely, by as much as 8 yr.

Age Validation for L226 and L302 Lake Whitefish

Of the 923 marked whitefish released in L226 during 1973 and 1974, 195 were recaptured in 1974 and 1975 for age comparisons. Of the 61 marked whitefish released in L302 in 1975, 29 were recaptured for age comparisons in autumn 1976. Results from the two separate basins of L302 were combined because the wall permitted intermixing of fish between basins. Results from the two basins of L226 are discussed separately because there was no interchange of fish between basins.

Comparisons of age at release and age at recapture for L226 NE showed that both aging methods were valid (Table 3). New annuli on both fin-rays and scales were very clear (Fig. 2). While on the average fin-ray aging gave more reliable results than scale aging, no bias was present in the number of recapture scale ages which were either greater or less than that predicted knowing the scale age at marking.

Table 3. Summary of differences in age of individual whitefish determined at initial marking (M) to later recapture (R) using the fin-ray and scale aging techniques. The number of individuals whose change in age (R–M) equaled the number of winters between marking and recapture is indicated by *.

Lake	Change in age (R–M)	No. recaptured 1 yr after release		No. recaptured 2 yr after release	
		Scales	Fin-rays	Scales	Fin-rays
L226 NE	−2 (≤)[a]	2	1		
	−1	1			
	0	8	1		
	1	42*	68*	7	1
	2	9	2	29*	37*
	3 (≥)[b]			3	
L226 SW	−2 (≤)	1	1		
	−1	3		1	
	0	13	1	6	
	1	34*	56*	21	2
	2	5	1	3*	23*
	3 (≥)	3		1	
L302	−2 (≤)		1		
	−1	3			
	0	19	10		
	1	2*	18*		
	2				
	3 (≥)				

[a]The age at recapture was 2 yr or less than at marking.
[b]The age at recapture was 3 yr or greater than at marking.

Comparisons of age at release and age at recapture showed that fin-ray aging was valid and scale aging questionable in L226 SW (Table 3). New annuli on fin-rays were very clear but new annuli on scales were frequently unclear or apparently absent. This was most pronounced in fish recaptured 2 yr after release (Fig. 3).

Comparisons of age at release and recapture in L302 showed that the fin-ray method was of questionable value and the scale method invalid (Table 3). New annuli on fin-rays were many times compressed to the old but were distinguishable in the majority of cases. New annuli on scales were rarely detected (Fig. 4).

Growth

Growth curves based on scale ages (Fig. 5) fell within the range reported for other lake whitefish populations (Healey 1975). Growth curves based on fin-ray ages could be extended to older age-groups more readily than those derived from scales and in many cases these indicated slower growth. The 11 popula-

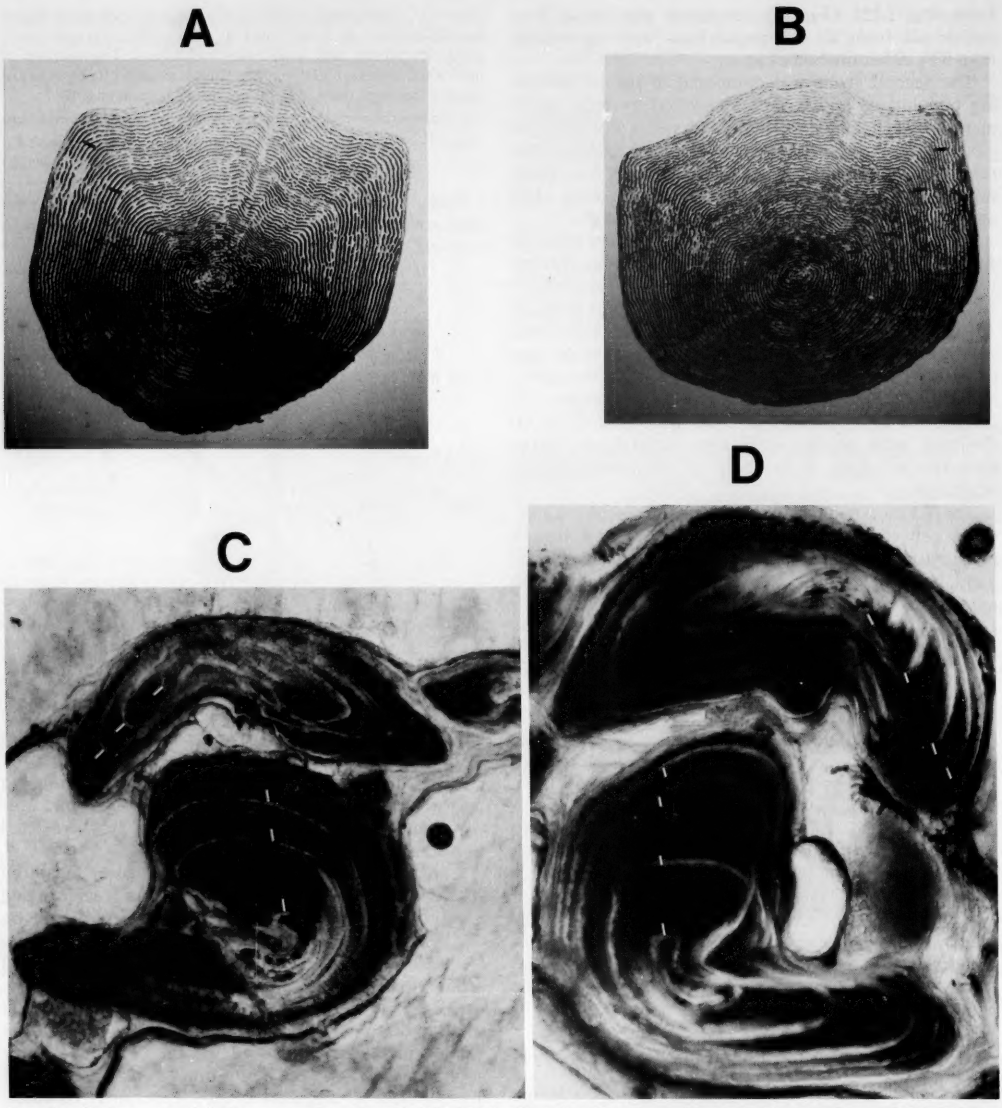

FIG. 2. Scales and pelvic fin-ray sections from a L226 NE whitefish taken at marking (A, C) and recapture (B, D) 1 yr later. Annuli are indicated by (–). Both scales and fin-ray sections were aged 3 at release and 4 at recapture.

tions could be divided into three groups based on comparisons between pairs of fin-ray and scale growth curves.

The first group was made up of four populations which had similar fin-ray and scale growth curves and were generally located in the upper range of whitefish growth (Fig. 5A). Three of these populations (Lake Huron, Lake Winnipeg, and Clay Lake) exhibited very rapid growth rates and contained larger individuals than other populations. The L122 population also showed good agreement between scale and fin-ray growth curves, but somewhat slower growth.

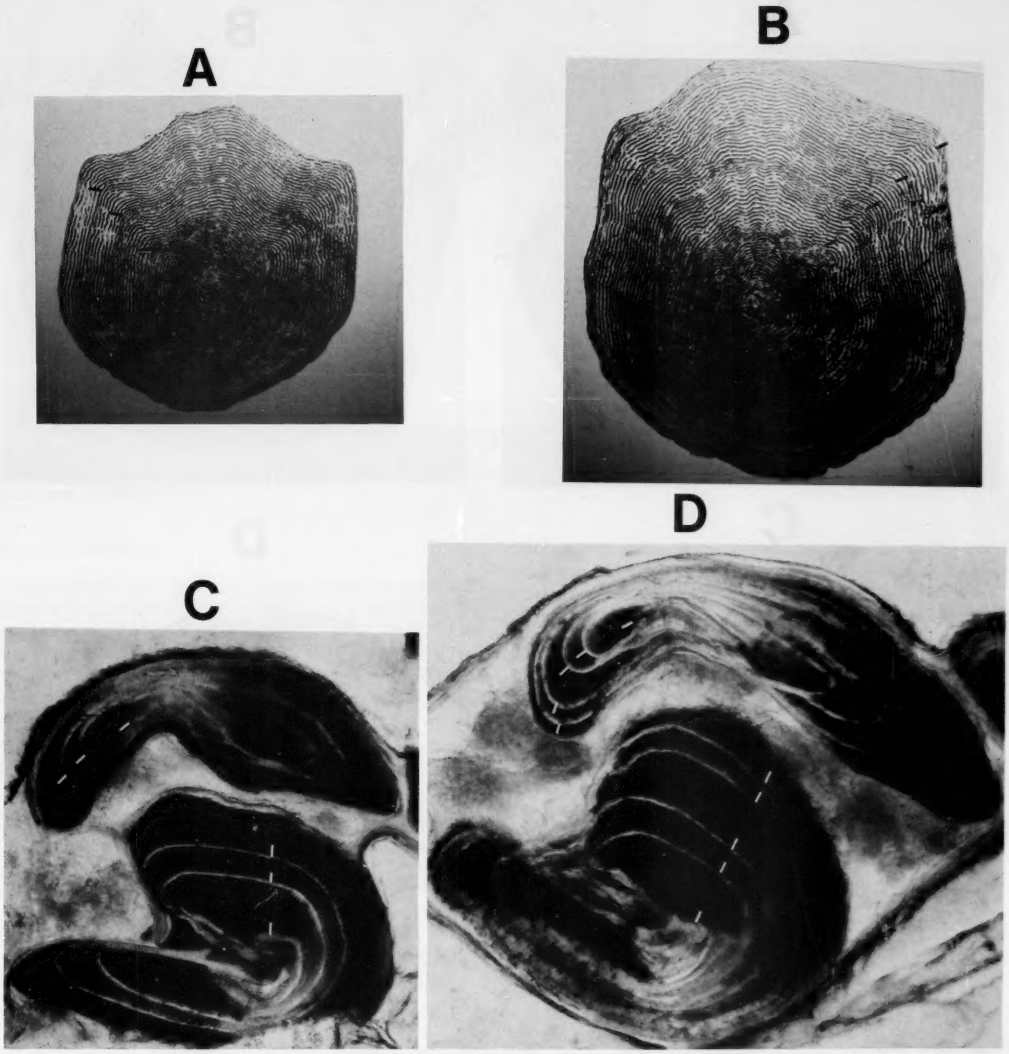

FIG. 3. Scales and pelvic fin-ray sections from a L226 SW whitefish taken at marking (A, C) and recapture (B, D) 2 yr later. Annuli are indicated by (–). Both scales and fin-ray sections were aged 3 at release. The fin-ray sections were aged 5 at recapture while the scales were aged 4.

The second group was made up of three populations whose fin-ray growth curves indicated moderately slower growth than those derived from scales (Fig. 5B). The scale growth curves for these populations, Southern Indian, L226 and L302, were generally in the middle range of whitefish growth. The L226 population (Fig. 6) is representative of this group and shows that differences between growth curves due to aging methods start at age 4, the age of sexual maturity for this population. Slower growth after sexual maturity using fin-ray ages was a common characteristic of this group.

The third group was made up of four populations whose fin-ray growth curves indicated much slower growth than those derived from scales (Fig. 5C). The

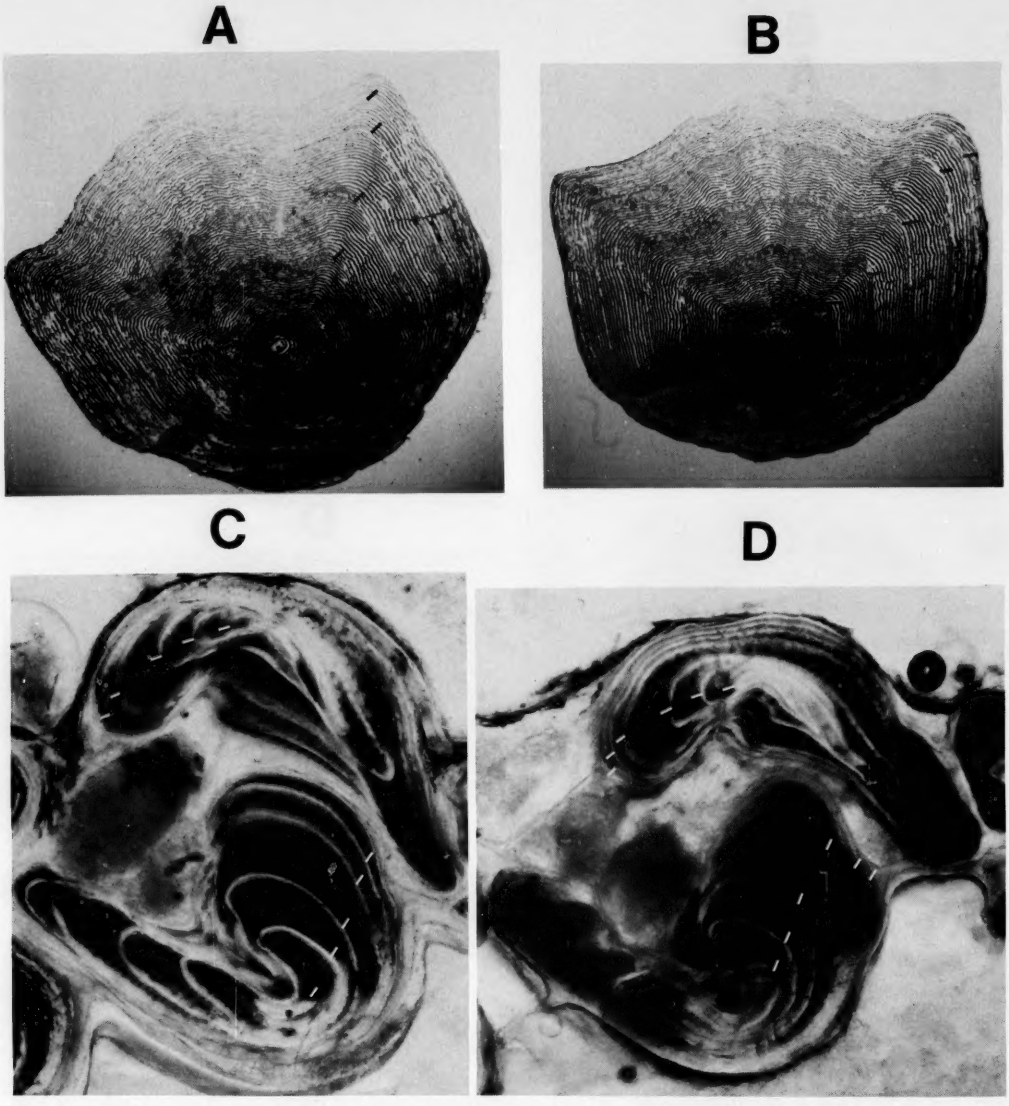

FIG. 4. Scales and pelvic fin-ray sections from a L302 whitefish taken at marking (A, C) and recapture (B, D) 1 yr later. Annuli are indicated by (–). Both scales and fin-ray sections were aged 5 at release. The fin-ray sections were aged 6 at recapture while scales were aged 5.

scale growth curves for these populations were generally in the lower range of whitefish growth. The Dezadeash, Opeongo, L310 and L468 populations had fin-ray curves that indicated much poorer growth than those derived from scales with differences starting in age-groups before sexual maturity. The Dezadeash Lake population, representative of this group (Fig. 6), showed that differences in growth curves can occur in fish as young as age 2, well before sexual maturity. These populations were composed almost entirely of small whitefish from many age-groups. Few individuals larger than 30 cm were present.

FIG. 5. A summary of individual comparisons between fin-ray and scale age based growth curves for 11 populations. A, the range of growth where fin-ray ages and scale ages resulted in very similar growth curves; B, the range where fin-ray age based curves differed from scale-based curves starting at age 4 or 5; C, the range where fin-ray age based curves differed from those of scales starting at age 2 or 3. Dots indicate the previously reported range of whitefish growth based on scale ages (Healey 1975).

ANNUAL SURVIVAL

Annual survival rates calculated from our data and previously published rates for the same populations are presented in Table 4 for 11 of the populations. Our catch curve rates and previously published rates based on scale ages agree well. For four populations survival estimates using fin-ray ages agreed well with corresponding scale estimates. In most populations fin-ray estimates were much higher than scale estimates. In only two cases was the scale based annual survival greater than its fin-ray counterpart. Generally, fin-ray survival rates were greater than those using scales and could be as much as twice the corresponding scale estimates. Two mark–recapture annual survival estimates independent of age data are available for comparison with catch curve rates. Both mark–recapture estimates are similar to fin-ray estimates for Lake Huron and L226. The scale survival estimate was similar to the mark–recapture estimate for Lake Huron, but well below the mark–recapture estimate for L226.

Discussion

WHITEFISH AGE DETERMINATION

A common method employed by many researchers to determine the level of confidence placed in a set of age determinations is to read the same set more than once, comparing ages for individual fish and reporting percentage agreement between sets of ages, or reaging individuals where disagreements occur. Ricker (1975)

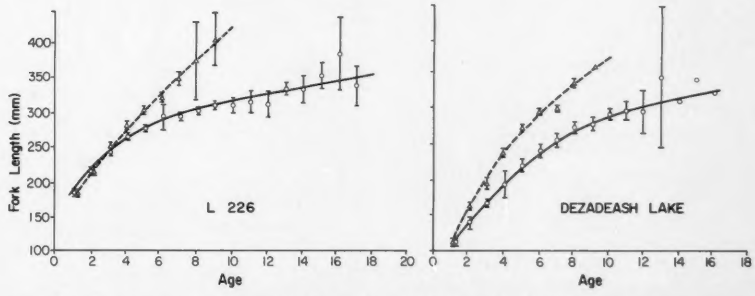

FIG. 6. Growth curves determined from fin-ray (○) and scale (△) mean lengths at age for Dezadeash Lake and L226. The dashed lines are eye-fitted growth curves based on scale ages, the solid lines based on fin-ray ages. Brackets represent the 95% confidence limits on each mean fork length at age.

TABLE 4. Annual survival rates for 11 whitefish populations using catch curves based on scale and fin-ray ages (K. H. M. unpublished data). The upper group is composed of populations undergoing moderate and heavy exploitation; the lower group of populations undergoing light exploitation or unexploited. Other independent estimates are presented for comparison where survivorship = 1 − total mortality rate.

	Annual survival			
Lake	Scale age ±95%	Fin-ray age ±95%	Scale literature values	Mark recapture
Huron	.17 ± .11	.19 ± .12	.07[a]–.38[b]	.11[f]
Clay	.46 ± .08	.48 ± .08		
122	.49 ± .05	.43 ± .06		
Winnipeg	.52 ± .06	.50 ± .06	.04–.50[c]	
468	.27 ± .10	.68 ± .08		
302	.35 ± .11	.64 ± .10		
Dezadeash	.44 ± .08	.60 ± .07		
Opeongo	.49 ± .07	.75 ± .05	.41–.59[d]	
226	.59 ± .04	.73 ± .04		.75[g]
Southern Indian	.60 ± .04	.70 ± .04	.30–.57[e]	

[a]Budd and Cucin (1962).
[b]Cucin and Regier (1965).
[c]Davidoff et al. (1973).
[d]Ricker (1947), dwarf form excluded.
[e]Ayles (1976).
[f]Spangler (1970).
[g]K. H. Mills unpublished data, Jolly–Seber estimate averaged for 3 yr data.

suggests that 80–90% agreement between sets is good. Only the results from the Lake Huron sample, the fastest growing population, exhibited this level of agreement between methods. Both Neth (1955) and Christie (1963) found close to 80% agreement between sets of scale ages for other relatively fast-growing populations. For slower-growing populations, we found agreement between methods or readers to be much lower. But even if agreement between sets of age determinations is good, it does not constitute validity for an aging technique. It only indicates consistency of the readers, clarity of the annuli, or both.

Comparisons of percent agreement between sets of age determinations can be an unreliable statistic with which to judge consistency between aging methods. For example, the L122 sample showed poor agreement between aging methods (30%) but rarely was the difference between aging methods greater than 1 yr. More importantly, there was little consistent bias between aging methods. The resulting growth curves and survivorships using either method of aging were almost identical. Despite the poor agreement between aging methods, the effects on parameters calculated from these ages was minimal. The L226 data showed better agreement between aging methods (47%) than the L122 data, but a consistent bias was present between the two aging methods. Fin-ray ages were generally greater than scale ages (Fig. 1). The resulting growth curves and survivorships for L226 whitefish showed large differences depending on which method of aging was used. Despite better agreement between aging methods in L226 than L122, there were great differences in the parameters calculated from the age data depending on which method of aging was used. Percent agreement only assesses whether ages determined by one method equal those of another, without quantifying the magnitude of differences.

Aging techniques for each population of interest should be validated in situ. This certainly is not possible in most cases. We suggest instead that scales and fin-rays be examined for a lake whitefish population to determine if one method consistently results in greater ages than the other. Simple data plots like Fig. 1 are more valuable for assessing aging accuracy than percent agreement between aging methods. A simple glance at Fig. 1 indicates whether there is a significant and consistent bias between the two methods, the magnitude of the bias, and at what age consistent differences first occur. Otoliths (Power 1978) or some other bony structure might also be used. Otolith ages for lake whitefish can agree well with fin-ray ages for some populations (K. Machniak, personal communication, Department of Fisheries and Oceans, Freshwater Institute), but it is necessary to kill fish to obtain otoliths and they are not as conveniently removed as a few fin-rays. The processes involved in deposition of scales are different from those of fin-rays or bone (Simkiss 1974). Therefore, an alternative method of age determination also has a physiological basis.

If consistent differences between fin-ray and scale ages occur, we believe the fin-ray ages are more dependable than scale ages. The different growth regimes in L226 and L302, combined with the age validation possible in these lakes, provide a plausible explanation for the aging disagreements found between different methods used for many lake populations. The productivity of L226 NE is much higher than that of L226 SW or L302 (Schindler and Fee 1974; Fee 1980). Marked whitefish grew faster in L226 NE (averaging 31 mm/yr), slower in L226 SE (16 mm/yr), and slowest in L302 (1 mm/yr). After a year of good growth, as in L226 NE, distinguishable annuli are formed on both scales and fin-rays. Under conditions of moderate growth, as in L226 SW, annuli are easily distinguishable on fin-rays, but not on scales. When growth is minimal, annuli are rarely distinguishable on scales, and not always apparent on fin-ray sections. Combinations of years of good, moderate, or minimal growth could easily explain the large differences in scale and fin-ray ages present in many of the lake whitefish populations of Table 2.

SOME IMPLICATIONS OF AGING ERRORS ON WHITEFISH GROWTH, SURVIVORSHIP, AND MANAGEMENT

Since fin-ray ages are correct more often than scale

ages, then when consistent differences occur between the methods, parameters based on fin-ray ages should be more reliable than those based on scale ages. The good agreement between the mark–recapture and catch curve survival estimates for L226 supports this (Table 4). This has important implications for lake whitefish management.

Carlander (1974) has shown that overestimates of growth often result from aging errors. Growth of populations in the middle and lower range of whitefish growth was overestimated using scales (Fig. 6). All of these populations were also unexploited except that of Southern Indian Lake, which is only lightly exploited (Ayles 1976). All these populations contained large numbers of older individuals, beyond age 8. While our data based on fin-ray ages are not as extensive as those based on scale ages for many whitefish populations, it is very likely that growth rates of other slower-growing lake whitefish populations have been overestimated using scales.

When scale and fin-ray ages differed markedly for a population, the annual survival based on fin-rays was higher than that using scales. Populations which showed the greatest difference between fin-ray and scale-based annual survival were the unexploited or lightly exploited populations (Table 4). All previous estimates of survivorship for unexploited populations are from scale age structure data (Healey 1975). It is very likely that these previous estimates were very conservative, underestimating the true rates.

When using fin-ray ages, an inverse relationship between growth and survivorship was apparent. Slow-growing populations had high survivorships. Fast-growing populations had low survivorships. If growth and survivorship were calculated using scale ages, no relationship was apparent. Healey (1975) also found little correlation between survivorship and growth in an extensive review of whitefish population dynamics based on scale ages. We believe that errors in aging whitefish using scales might have obscured this relationship.

While our data show that scale age determinations are acceptable for moderately or heavily exploited populations, and unacceptable for lightly or unexploited populations, others which are in transition from relatively unexploited to heavily exploited could also show errors due to aging. Current management practice for northern lake whitefish fisheries is to crop the populations more intensively. Many of these populations exhibit relatively slow growth rates. There is sufficient evidence to suggest that an increase in harvest will produce an increase in growth rate for lake whitefish (Healey 1975). The use of scale ages instead of fin-ray ages for populations such as L226 or Dezadeash Lake (Fig. 6) before and after cropping could result in false conclusions about the effects of increased cropping. While scale ages might show an increase in length at age for young age-groups after increased harvest, the full impact on older age-classes might be lost because larger sized, initially underaged fish were removed (Fig. 7A). Similarly, there is sufficient evidence to suggest lake whitefish respond to increased cropping by a decline in age at maturity (Healey 1975). This could also be obscured by errors from scale ages (Fig. 7B).

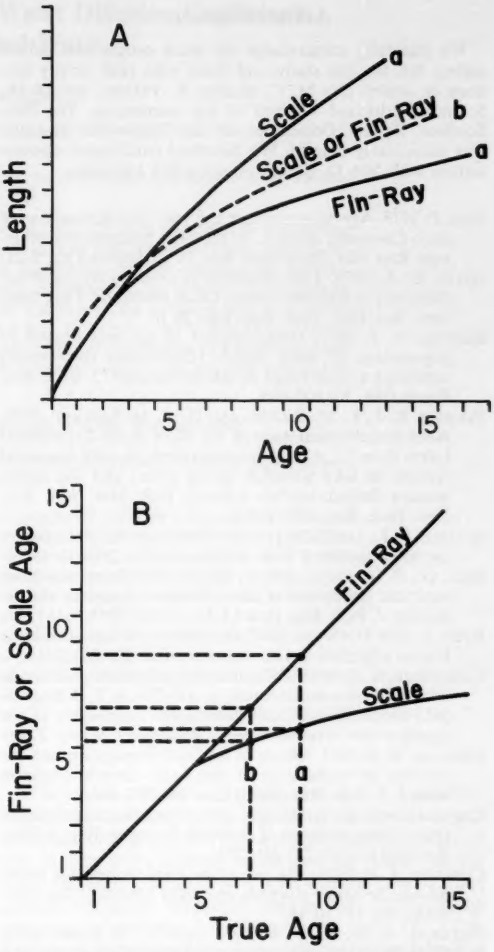

FIG. 7. Some possible consequences of incorrect scale aging under conditions of changing exploitation: (A) growth curves and (B) age at sexual maturity. Condition before exploitation (a) and after (b).

If we are to have predictive management of lake whitefish and other fish populations, we must recognize that significant, widespread aging errors are possible and that these errors can introduce significant bias in parameters used for management decisions.

Acknowledgments

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In Situ Measurements of Pore Water Diffusion Coefficients Using Tritiated Water

R. H. HESSLEIN[1]

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York, N.Y. 10964, USA

HESSLEIN, R. H. 1980. In situ measurements of pore water diffusion coefficients using tritiated water. Can. J. Fish. Aquat. Sci. 37: 545–551.

In situ diffusion coefficients in the pore waters of Lake 227 were measured by monitoring the movement of tritiated water into the pore waters. The diffusion coefficients were determined by analytical and numerical modeling of the tritium profiles. During the summer, tritiated water diffuses into the sediment by molecular diffusion and this process is well approximated by a constant diffusivity model. The summer coefficients for diffusion were $1–2 \times 10^{-5}$ $cm^2 \cdot s^{-1}$ at a water depth of 1.5 m (20°C) and $0.3–0.8 \times 10^{-5}$ $cm^2 \cdot s^{-1}$ at 8.75 m (4°C). During fall overturn, clear evidence was found for enhanced mixing of pore waters to a sediment depth of 10 cm at water depths of 0.75 and 3.85 m. This enhanced mixing was not accompanied by mixing of the solid phase of the sediments.

Key words: sediments, pore water, diffusion coefficients, tritium, lake sediments

HESSLEIN, R. H. 1980. In situ measurements of pore water diffusion coefficients using tritiated water. Can. J. Fish. Aquat. Sci. 37: 545–551.

Par contrôle du mouvement d'eau tritiée dans les eaux de pore, on a mesuré les coefficients de diffusion in situ de ces eaux dans le lac 227. Les coefficients ont été déterminés par modélisation analytique et numérique des profils de tritium. En été, l'eau tritiée se diffuse dans le sédiments par diffusion moléculaire et l'on obtient une bonne approximation du processus avec un modéle de diffusivité constante. Les coefficients de diffusion en été sont de $1–2 \times 10^{-5}$ $cm^2 \cdot s^{-1}$ à une profondeur d'eau de 1,5 m (20°C) et $0,3–0,8 \times 10^{-5}$ $cm^2 \cdot s^{-1}$ à 8,75 m (4°C). On a une claire indication, lors du brassage général d'automne, qu'il y a plus grand mélange des eaux de pore à une profondeur de sédiments de 10 cm à des profondeurs d'eau de 0,75 et 3,85. Ce mélange plus intense n'est pas accompagné d'un mélange de la phase solide des sédiments.

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DIFFUSION coefficients in sediment pore waters are necessary to calculate fluxes of dissolved materials though the sediments. These fluxes are useful in understanding the importance of sediment contribution to processes in the overlying water and in describing sediment diagenetic processes. Both theoretical and laboratory estimates of the pore water diffusion coefficients have been made in the past (Berner 1975; Guinasso and Schink 1975; Li and Gregory 1974). The theoretical approaches have in various ways applied the corrections of porosity and tortuosity to free-media diffusion. Laboratory measurements have been done on a number of porous media in diffusion cells using a variety of dissolved compounds. The estimates of diffusion coefficients from these approaches have in some cases been supported by observations in the field of distributions of dissolved substances in pore water. In situ measurements of diffusion coefficients in pore water have been rare.

Whole-lake additions of tritiated water to Lake 227, Experimental Lakes Area, northwestern Ontario, provided an ideal opportunity to make measurements of the in situ self-diffusion coefficient for water in the L227 sediments. Tritium was added to the lake in three distinct injections; the mixed layer on July 28, 1975, the thermocline and the hypolimnion on July 13, 1975. The vertical distribution of the tritium in the lake was monitored routinely for the mixing experiments (Quay 1977; Quay et al. 1980). Measurements in the sediments were made at several times after the injection at various depths to determine the speed of the self-diffusion of water and to observe any enhanced mixing due to bioturbation or turbulent events associated with changes in weather or fall lake overturn. In addition, cores were taken during the summer and after fall overturn was in progress (Kipphut 1978) to determine how mechanical mixing, if it was apparent, affected the solid portion of the sediment.

Lake 227 is a small lake on the Canadian Shield near Kenora, Ontario. It has a surface area of 5 ha, a maximum depth of 10 m and a mean depth of 4 m. The physics, chemistry, and biology of Lake 227 have been extensively discussed over the last 10 yr. For more

[1]Present address: Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6.

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information see *J. Fish. Res. Board Can.* 28(2) (1971), 30(10) (1973), and Schindler and Fee (1974).

Methods

The methods used to add the tritium to Lake 227 are covered in detail in Quay (1977) and Quay et al. (1980). The results of the lake mixing experiments are also discussed in detail in these papers and will be used here only where necessary to describe sediment processes.

Sampling for tritium measurements was done with the pore-water peeper (Hesslein 1976a). This sampler consists of many compartments machined at 1-cm intervals in a sheet of acrylic 10 × 60 cm. The compartments are covered by a dialysis membrane. The sampler is inserted into the sediments by a diver and water in the compartments allowed to passively equilibrate with the pore-waters for about 10 d before removal for analysis. A 1- or 2-mL aliquot was taken from each sample, and 14 mL of PCS (Amersham Searle) added to it in a polyethylene liquid scintillation vial, and it was counted in a liquid scintillation counter (Nuclear-Chicago Unilux II or Picker Nuclear Liquimat 220). The counting efficiency was about 34% with a background level of 20 cpm in the N–C machine and 22% and 12 cpm, respectively, in the Picker machine. Samples were normally counted for 40–100 min depending on the tritium leevl. This resulted in a total count of 1000–7000. The overall coefficient of variation in sampling and counting is probably ±5%. Standards were prepared by adding a 10-μL known "hot" tritium spike to a previously counted sample and recounting after mixing the standard well.

Cores were taken by a "slingshot" piston corer similar to one described by Murray (1976), for use in soft sediments. It consists of a clear acrylic core barrel with a steel and aluminum frame to guide the barrel. Force is applied by two stretched loops of rubber tubing and the trigger is released by the arrival of the messenger weight. The operation of the corer is very rapid. It is light and easy to work with in the field and in most cases, in soft sediments, takes excellent cores with relatively undisturbed interfaces. The average core is about 45 cm long. After the cores were retrieved they were extruded and sectioned into preweighed tins, weighed, dried to 110°C, and reweighed to calculate percent water. The dried sediment was then homogenized and placed in plastic 5 cm diameter petri dishes for counting of Cs^{137} by means of a Germanium-Lithium crystal gamma spectrometer (Princeton Gamma Tech). The concentration of Cs^{137} is expressed in pCi/g (1 pCi = 37 KBq) of dry sediment relative to a standard prepared in similar sediment and of similar geometry.

Theory

The diffusion of tritiated water into the sediments from a source of constant concentration is described by the equation $C = C_0 \operatorname{erfc} z/2\sqrt{Dt}$, where C is the concentration of tritium at depth z into the sediments (Carslaw and Jaeger 1959). C_0 is the concentration of tritium at $z = 0$. D is the diffusion coefficient of tritiated water in water and t represents time. Because of the stochastic nature of the changes in tritium concentration in the overlying water due to vertical mixing in the lake (Quay 1977), this model could not be directly applied. Carslaw and Jaeger (1959) give and equation describing the distribution with depth of a quantity whose concentration at $z = 0$ is a step function. This results in a series of terms of the type shown above. For a semi-infinite body of initial concentration zero we have C at $z = 0$ as C_0 for $0 < t < T$, C_1 for $T < t < T_1$, C_2 for $t > T_1$, and so on. For three terms then:

$$C = C_0 \operatorname{erfc} \frac{2}{2\sqrt{Dt}} + (C_1 - C_0) \operatorname{erfc} \frac{z}{2\sqrt{D(t-T)}} + (C_2 - C_1) \operatorname{erfc} \frac{z}{2\sqrt{D(t-T_1)}}$$

The first term describes diffusion under the effect of C_0 from 0 to t. The second term describes the additional diffusion due to the added amount over the period T to t as dictated by $(C_1 - C_0)$ and so on. To avoid the tedious use of tables of erfc the values were numerically approximated by two series (Carslaw and Jaeger 1959). For $x \leq 1.65$

$$\operatorname{erfc}(x) = 1 - \frac{2}{\sqrt{\pi}} \sum_{n=0}^{\infty} \frac{(-1)^n x^{2n+1}}{(2n+1)n!}$$

and for $x > 1.65$

$$\operatorname{erfc}(x) = \sqrt{\pi} \exp\left(-x^2\left(\frac{1}{x} - \frac{1}{2x^3} + \frac{1\cdot 3}{2^2x^5} - \frac{1\cdot 3\cdot 5}{2^3x^9} + \ldots\right)\right)$$

1.65 was found to be a convenient switchover point. The first series was carried to $n = 10$, the second to the four terms shown in the parentheses. These approximations give an error in estimation of erfc of less than 0.1% of the surface concentration at a depth of 50 cm.

A numerical approach was used to construct a two-zone model for the diffusion of tritium into the pore waters. This model was based on the equation

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial z^2}$$ which in finite difference form is:

$$C_{t_2} = D\left(\frac{C_1 - C_2}{z^2} - \frac{C_2 - C_3}{z^2}\right)t + C_{t1}$$

with all coefficients as previously defined. The concentration at the sediment–water interface could be specified at any time as well as the value and depth range covered by the diffusion coefficient for each zone. The model used had 50 layers at 1-cm intervals.

Because of the finite equilibration time required by the sampler and the changing concentrations in the pore waters a true equilibrium is not reached in the sample compartments. This effect could be built into the analysis if the time course of equilibration were well known. This is, however, a function of the type of sediment (Hesslein 1976b). For this experiment a date 3 d before removal of the sampler was chosen as being represented by the measured profile.

Results

The composition of the sediments in a core taken at 1.5 m depth in August 1975 (Table 1) shows a mean water content of 96.25% with very little change over the 45 cm length of the core. Carbon and nitrogen con-

TABLE 1. Composition of core taken from 1.5 m depth in Lake 227, August 1975.

Depth interval (cm)	% Water	Microgram/Milligram dry weight	
		Carbon	Nitrogen
From 0 to			
2.21	95.9	255	23.5
5.22	96.2	248	19.0
7.65	96.5	246	19.0
9.87	96.9	253	19.5
11.87	96.8	248	19.0
14.13	96.8	257	19.5
16.50	96.7	252	20.5
18.97	96.5	250	19.0
21.41	96.4	254	21.0
23.76	96.4	243	18.5
26.21	96.3	261	19.0
28.87	96.2	253	19.5
31.21	96.2	250	19.0
33.42	96.2	249	19.0
35.84	96.1	242	19.0
38.32	96.1	256	20.0
40.64	95.9	262	19.0
43.05	95.3	241	18.5
45.43	95.4	248	21.5
Mean values	96.25	251	19.6
±95% confidence interval	0.86	12	2.5

tent of the solid phase are also very constant throughout the core.

The concentrations of tritium with time at water depths of 0.75 and 8.75 m measured with liquid scintillation techniques (Quay 1977) are shown in Fig. 1, with the step functions used to approximate the values. Quay (1977) estimates the error of the tritium measurements to be ±8%. The choice of step functions used to represent the concentrations was based on averages of groups of values which had low variance or

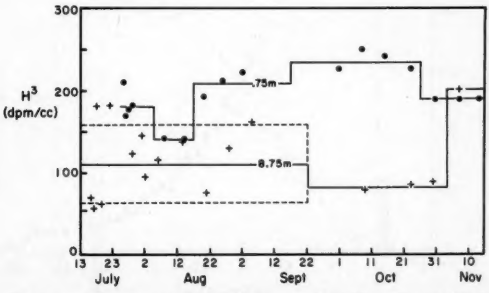

FIG. 1. Tritium concentrations in Lake 227, 1975 at depth of 0.75 and 8.75 m with step function approximations used in modeling. One standard deviation above and below the average for the first step at 8.75 m are shown by dotted lines.

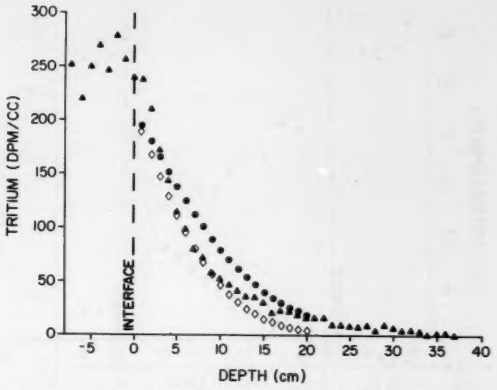

FIG. 2. Tritium in Lake 227 pore water, 0.75 m, September 1975, ▲. Model generated profiles: $D = 1.0 \times 10^{-5}$ cm$^2 \cdot$s^{-1}, ◇; $D = 2.0 \times 10^{-5}$ cm$^2 \cdot$s^{-1}, ●.

were separated widely in time. Steps were assigned to a time half way between group end point times. This worked quite well at a depth of 0.75 m. The high variance in the earlier data at 8.75 m is due to both horizontal and vertical gradients in tritium, as well as internal wave activity, which moves the water relative to sediments (Quay 1977). The average value to September 5 is given, as well as one standard deviation in each direction. Later values show little variability as the gradients in the water column have decreased with time (Quay 1977).

The concentration of tritium at any level in the thermocline was too erratic as a function of time to be approximated in this way. This was a result of the high time and spatial variance due to vertical and horizontal mixing (Qua 1977; Quay et al. 1980). The inability to define the concentration at the interface as a function of time prevents the use of the model as it must be forced by the concentration at the interface. All tritium profiles in the sediments are shown in Fig. 2 to 9, including those which were not modeled. The two modeled depths, 0.75 and 8.75 m are shown with the model results for various values of D.

The three profiles from the summer (3.6, 4.2, 0.75 m) exhibit shapes expected to result from more of less uniform diffusion rates through the sediments with a constant source value. There is no indication of a high rate of mixing in the top few centimetres of the sediment. The profile for September 5–9, 0.75 m, falls between the model profiles for sediment diffusion coefficients of 1 and 2×10^{-5} cm$^2 \cdot$s^{-1}. This is what would be expected if diffusion in these sediments proceeded only by molecular processes. The self-diffusion coefficient of water at 20°C is 2.1×10^{-5} cm$^2 \cdot$s^{-1} (Wang et al. 1953). The correction for porosity is only 5% (>95% water content, Table 1) and tortuosity, though not easily estimable, is also probably small. This is

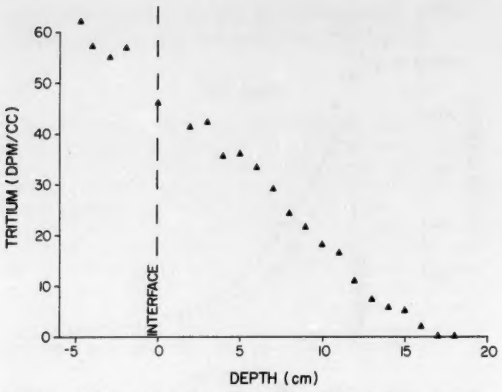

FIG. 3. Tritium in Lake 227 pore water, 3.6 m, August 1975.

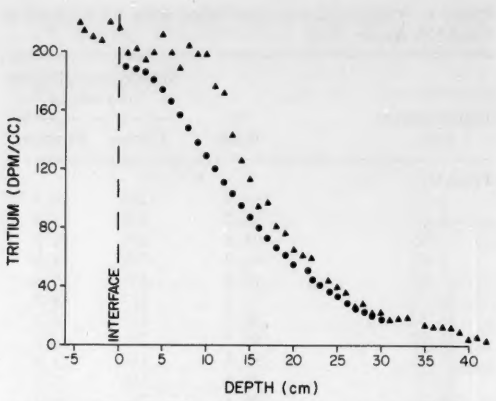

FIG. 5. Tritium in Lake 227 pore water, 0.75 m, October 1975, ▲. Model generated profile: $D = 2 \times 10^{-6}$ cm$^2 \cdot$s^{-1}, ●.

well within the error of the determination. In both the 3.6 and 4.2 m profiles the distance over which the concentration drops by a factor of 2 is on the same order as in the 0.75 m profile, suggesting that sediment diffusion coefficients at these depths are not too different from those at 0.75 m.

The tritium profiles for the sediments at water depths of 0.75 and 3.85 m (Fig. 5 and 8) in the fall show a definite zone of constant concentration to a depth of about 10 cm. Model profiles using a constant diffusion coefficient with time and depth are shown with the 0.75 m profile. Clearly a higher mixing rate has prevailed over the top 10 cm of the sediments for at least a short time. The profile approaches the model profile for 2×10^{-5} cm$^2 \cdot$s^{-1} at depth suggesting that diffusion below 10 cm depth has continued by molecular processes. There is no way to accurately estimate rate of mixing in the upper 10 cm zone while the lower was held at 2×10^{-5} cm$^2 \cdot$s^{-1}. The data can be fitted by model profiles for 5×10^{-5} cm$^2 \cdot$s^{-1} applied for 3 d prior to sampling. As the cause of this increased mixing of the top 10 cm of pore water is unknown, it is difficult to determine the correct time interval over which it took place. These model-generated profiles are shown with the data in Fig. 6 and 7. The tritium diffusion into the sediments at 3.85 m was not modeled, as previously explained, but the profile (Fig. 8) exhibits the same shape as that at 0.75 m. Since the 3.85 m level had been part of the mixed layer of the lake for at least 30 d, it is likely that the same process has affected the pore water mixing at 0.75 and 3.85 m. It

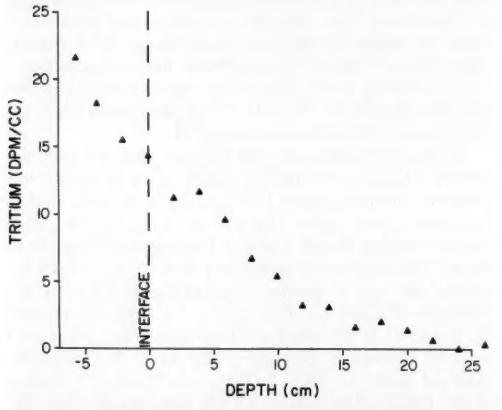

FIG. 4. Tritium in Lake 227 pore water, 4.2 m, August 1975.

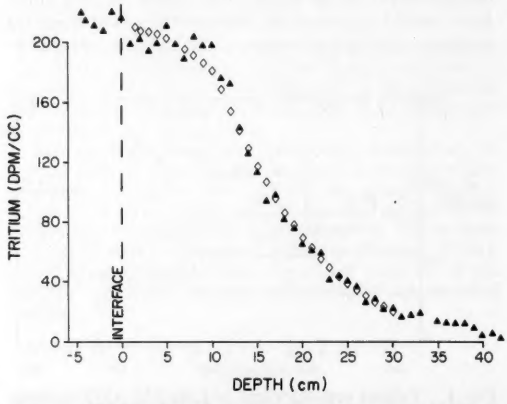

FIG. 6. Tritium in Lake 227 pore water 0.75 m, October 1975, ▲. Model generated profile: $D = 2 \times 10^{-5}$ cm$^2 \cdot$s^{-1} to September 30 then 5×10^{-5} cm$^2 \cdot$s^{-1} from 0 to 10 cm and 2×10^{-5} cm$^2 \cdot$s^{-1} from 10 to 50 cm until October 27, ◇.

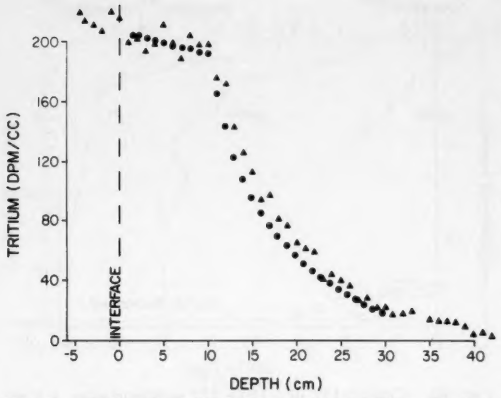

FIG. 7. Tritium in Lake 227 pore water, 0.75 m, October 1975, ▲. Model generated profile: $D = 2 \times 10^{-5}$ cm$^2 \cdot$s^{-1} to October 24 then 50×10^{-5} cm$^2 \cdot$s^{-1} from 0 to 10 cm and 2×10^{-5} cm$^2 \cdot$s^{-1} from 10 to 50 cm until October 27, ●.

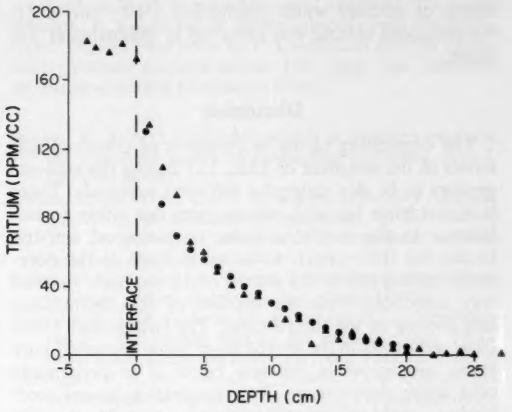

FIG. 9. Tritium in Lake 227 pore water, 8.75 m, October 1975, ▲. Model generated profile: $D = 0.5 \times 10^{-5}$ cm$^2 \cdot$s^{-1}, ●.

should be mentioned that the homogeneous nature of the top 10 cm in both profiles is not entirely due to mixing in the pore water. The concentration in the overlying water decreased over the last few days before sampling (Fig. 1) due to entrainment of previously isolated deepwater low in tritium as the lake became isothermal. This is taken into account in the modeling of the 0.75 m profile by the last time step of the model.

In contrast with the pore water tritium profiles, the profile at 8.75 m in late October shows no homogeneous layer. A profile generated by the analytical model using the mean value at the interface to September 5 is shown with the profile in Fig. 9. The data fit best (by least squares comparison) the model-generated profile using a diffusion coefficients of 0.3×10^{-5} cm$^2 \cdot$s^{-1} using the mean value minus one standard deviation and 0.4×10^{-5} cm$^2 \cdot$s^{-1} using the mean value plus one standard deviation to September 5. These values are less than the diffusion coefficient of tritiated water in water at 4°C of 1.3×10^{-5} cm$^2 \cdot$s^{-1} (Wang et al. 1953). Again, as at 0.75 m, the correction for porosity and tortuosity is ~5%. There are two complicating factors in the interpretation of the data at 8.75 m. First, the concentration of tritium in the overlying water changed drastically very shortly before the peeper was removed. The uncertainty of the time of the change (see Fig. 1) creates considerable uncertainty in the truth of the models in the upper few centimetres. In addition, observation of cores showed the sediments at this time at 8.75 m to be characterized by an extremely loose flock-like layer of algal material about 3–8 cm thick. Whether this layer was present throughout the season or recently deposited is unknown. Since a weight and platform rig rather than diver was used to place this peeper, and the interface determined by the position of the platform, it is possible that some compression of this layer occurred, thus displacing upward the apparent depths of the data points. In any case, the profile indicates no major deviation from that form expected by molecular processes alone.

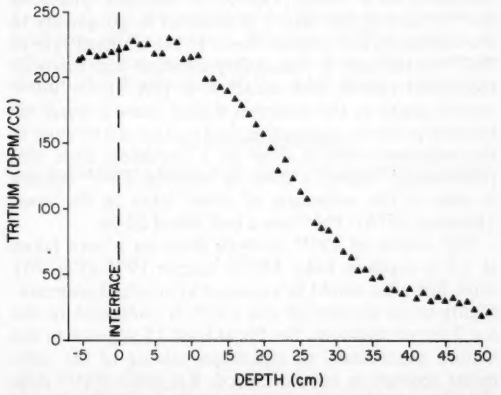

FIG. 8. Tritium in Lake 227 pore water, 3.85 m, October 1975, ▲.

In summary, the modeling of the tritium profiles yields diffusion coefficients for tritiated water of $1–2 \times 10^{-5}$ cm$^2 \cdot$s^{-1} throughout the epilimnion sediment column during the summer. During fall mixing an undetermined but higher diffusion coefficient prevails in the top 10 cm of the sediments formerly overlain by warm (>10°C) water. The deeper (>10 cm) part of these sediments still exhibit low ($1–2 \times 10^{-5}$ cm$^{2 \cdot -1}$) mixing rates during fall overturn. The modeling of tritium movement in the 8.75 m sediments yields a diffusion rate in the sediments which is less than the molecular dif-

fusion of tritiated water (about 0.5×10^{-5} cm$^2 \cdot$s^{-1}). No enhanced mixing was observed in sediments at this depth.

Discussion

The controlling factor in transport of dissolved materials in the sediment of Lake 227 during the summer appears to be the molecular diffusion processes. There is no evidence for high mixing rates due either to turbulence in the overlying water or biological activity. In the fall there seems to be an increase in the pore-water mixing rate in the top 10 cm of sediment in some way associated with the erosion of the thermocline and cooling of the mixed layer. The two profiles taken from sediments in the mixed layer show increased pore water mixing while the one below it in perennially cold water shows none. Two possible processes seem likely as explanations of this phenomenon. There could be an increase in turbulence in the mixed layer as it deepens and cools and is acted on by the more vigorous fall winds. If this is true, it is difficult to see why there is no evidence of even slightly enhanced mixing in the sediments in contact with the summer mixed layer.

The other possible explanation for the enhanced mixing is that a convective overturn occurs in the pore waters. The temperature of the pore waters at the end of the summer season can be calculated with the equations presented for the tritium diffusion model. As the coefficient for the molecular transport of heat in water is 1.2×10^{-5} cm$^2 \cdot$s^{-1}, heat penetrates the sediments at a far greater rate than tritium. By the end of August the temperature at 30 cm depth in sediments in contact with the mixed layer is above 18°C. This has been verified by some measurements with thermistors (P. D. Quay and R. H. Hesslein unpublished data). If the overlying water cools rapidly, its density may rise above that of the still warm pore waters and an unstable condition will be created. The degree of this instability will depend on the rate of cooling of the overlying water, the temperature gradient in the pore water, and the additional stability imposed by the considerable gradients in dissolved species in the pore waters (Hesslein 1976b). The density which can be attributed to dissolved chemical species is less than that due to a change of 1°C (at 18°C) in the pore water. A rapid change in the lake water of >1°C would cause a density instability relative to all pore water to at least 30 cm depth. The degree of instability required for mixing or replacement of pore waters is not known. A number of theoretical investigations have been made in the stability of fluids in porous media. Cambarnows and Bories (1974) have reviewed the recent literature. Other field data suggesting substantial interstitial water replacement have been present by Thorstenson and Mackenzie (1974).

Knowing whether or not the sediments themselves are mixed during this process would be very valuable in understanding these mixing phenomena. Fortunately

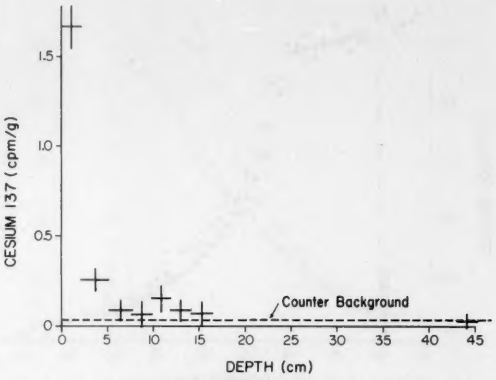

FIG. 10. Cesium-137 in a Lake 227 sediment core, 1.5 m, August 1975; 95% confidence interval for counting is shown. The absolute efficiency of the counter was ~1.6%.

there is some information available on the mixing of the sediments. Measurement of Cs^{137} distribution in the sediment has been done as part of this study and by George Kipphut on a core from a depth of 9 m in Lake 227 in another study (Kipphut 1978). Kipphut has also measured sedimentation rates by Pb^{210} activity. Both Cs^{137} and Pb^{210} have generally been assumed to remain with the solid fraction of the sediments. Cs^{137} was introduced to the Earth's atmosphere by nuclear testing, primarily in the years 1960–65. It has thus recorded a 10–15 yr history of mixing in the Lake 227 sediments. According to Kipphut's estimate (Pb^{210}) of the sedimentation rate in Lake 227 of 0.1 cm$^{-1} \cdot$yr^{-1}, the Cs^{137} would, under conditions of no mixing in the sediment, be confined to the top 1–1.5 cm of sediment. Kipphut's estimate was made for a depth of 9 m and may be an overestimate for 1.5 m due to sediment focusing. Pb^{210} enters the lake primarily from the atmosphere as a decay product of naturally produced Rn^{222}. Once in the lake it is believed to go quickly to the sediments and remain there. If the addition rate of Pb^{210} to the lake is reasonably constant and the sedimentation rate is also constant, a plot of Ln Pb^{210} against depth in the sediment should show a linear relationship due to radioactive decay. Any mixed zone in the sediments should show as a deviation from this relationship. Indeed a zone of uniform Pb^{210} activity is seen in the sediments of other lakes in the area. (Kipphut 1978). Pb^{210} has a half-life of 22 yr.

The results of Cs^{137} analysis done on a core taken at 1.5 m depth in Lake 227 in August 1975 (Fig. 10) show just what would be expected in unmixed sediment. Eighty-seven percent of the Cs^{137} is contained in the top 2 cm of sediment. So, for at least 15 yr prior to the tritium experiment, no significant mixing of the sediments appears to have occurred. Kipphut's Pb^{210} data also consistently reject the idea of a mixed layer of depth greater than 2 cm (Fig. 11). The similarity in

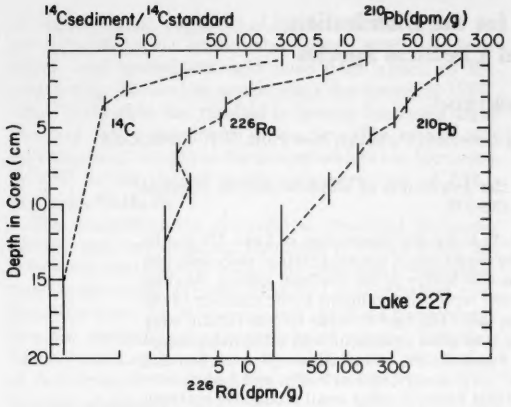

FIG. 11. Radium-226, carbon-14, and lead-210 in a Lake 227 sediment core. (Redrawn with permission from G. Kipphut [1978].)

both the distribution and integrated amount of Cs^{137} in the 1.5 m core presented here and Kipphut's 9 m core give some confidence that the sedimentation and mixing rates are comparable. Emerson (1974) presented data on the distribution of artificially introduced Ra^{226} in the Lake 227 sediments. He shows a penetration into the sediments of Ra^{226}, introduced into the lake 3 yr before, in 1961, of up to 6 cm and suggests that this is an indicator of mixing of the sediments. The stability of Ra^{226} on the solid phase of the sediments, especially in a reducing environment, is not nearly so certain as that of Cs^{137} and Pb^{210}. It is possible that some migration of the Ra^{226} occurred during the 3 yr. In any case, the 6 cm mixing depth suggested by Emerson for a 3-yr history is considerably less than the 10 cm in less than 1 mo shown by the tritium. The process responsible for the more rapid mixing of tritium in the top 10 cm of the sediments (which is also demonstrated by smoothing of chemical gradients in pore waters, Hesslein 1976b) appears to affect only the pore waters and does not appear to disturb the distribution of the solid phase of the sediment.

Acknowledgments

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Whole-Lake Model for the Distribution of Sediment-Derived Chemical Species

R. H. HESSLEIN[1]

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York, N.Y. 10964, USA

HESSLEIN, R. H. 1980. Whole-lake model for the distribution of sediment-derived chemical species. Can. J. Fish. Aquat. Sci. 37: 552–558.

The distribution of CH_4, ΣCO_2, and NH_3-N below the thermocline of Lake 227 can be reproduced using a simple numerical model. The model uses a constant vertical eddy diffusion coefficient and assumes the sediments to be the sole source of the chemical species. Analogs dependent on oxygen concentration can effectively represent conditions at the interface of the modeled region and the shallower depths in the lake. The best fit value for the vertical eddy diffusion coefficient is 3.1×10^{-3} cm$^2 \cdot$s^{-1}. This is in good agreement with other independent measurements of this phenomena. The best fit fluxes of CH_4, dissolved inorganic carbon and NH_3-N are respectively: 13×10^{-3} mol$\cdot$m$^{-2}\cdot$d^{-1}, 7.5×10^{-3} mol$\cdot$m$^{-2}\cdot$d^{-1}, and 1.65×10^{-3} mol$\cdot$m$^{-2}\cdot$d^{-1}. The flux of methane is similar to that found in other small productive northern lakes and the ratio of CH_4:ΣCO_2 production of 1.73 falls in the range of values established for fermentation of mixed organic materials and sediments. The flux of ΣCO_2 is 50–75% of the bicarbonate flux ($10–15 \times 10^{-3}$ mol$\cdot$m$^{-2}\cdot$d^{-1}) found previously in Linsley Pond, Connecticut.

Key words: sediment–water interactions, methane, CO_2, and ammonia fluxes, diffusion from sediments, Experimental Lakes Area

HESSLEIN, R. H. 1980. Whole-lake model for the distribution of sediment-derived chemical species. Can. J. Fish. Aquat. Sci. 37: 552–558.

Il est possible, à l'aide d'un modèle numérique simple, de reproduire la répartition de CH_4, ΣCO_2 et NH_3-N au-dessous de la thermocline du lac 227. Le modèle utilise un coefficient de diffusion tourbillonnaire verticale constant et suppose que les sédiments sont l'unique source des espèces chimiques. Des analogiques dépendant de la concentration d'oxygène peuvent très bien représenter les conditions à la couche limite entre la région modélisée et les plus faibles profondeurs du lac. La meilleure valeur pour le coefficient de diffusion tourbillonnaire verticale est de $3{,}1 \times 10^{-3}$ cm$^2\cdot$s^{-1}. Ceci s'accorde bien avec d'autres mesures indépendantes de ce phénomène. Les meilleurs valeurs pour les flux de CH_4, carbone inorganique dissous et NH_3-N sont respectivement : 13×10^{-3} mol$\cdot$m$^{-2}\cdot$jour^{-1}, $7{,}5 \times 10^{-3}$ mol$\cdot$m$^{-2}\cdot$jour^{-1} et $1{,}65 \times 10^{-3}$ mol$\cdot$m$^{-2}\cdot$jour^{-1}. Le flux de méthane est semblable à celui trouvé dans d'autres petits lacs septentrionaux productifs, et le rapport de production CH_4:ΣCO_2 de 1,73 tombe entre les limites établies pour la fermentation d'un mélange de matériaux organiques et de sédiments. Le flux de ΣCO_2 est de 50 à 75% de celui du bicarbonate ($10–15 \times 10^{-3}$ mol$\cdot$m$^{-2}\cdot$jour^{-1}) observé antérieurement dans l'étang Linsley, dans le Connecticut.

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THE flux of nutrients from sediments and their consequent distribution in the water is an essential part of the nutrient cycles in lakes. This paper presents a numerical model of the sediment flux-water mixing system which controls the concentrations of methane (CH_4), total dissolved inorganic carbon (ΣCO_2), and nitrogen as ammonia (NH_3-N) below the euphotic zone of Lake 227 in northwestern Ontario. The distributions of these species coupled with eddy diffusion in the thermocline determine the fluxes of carbon and nitrogen to the epilimnion in summer (Hesslein and Quay 1973; Schindler et al. 1977). The mass of carbon present in the lake as CH_4 and ΣCO_2 at fall overturn plays an important role in determining the ability of the lake to accumulate carbon (Schindler et al. 1973). Modeling the sediment flux and distribution of these chemical species can also provide a check on studies on the intensity of microbial activity in the sediments (Robertson 1978).

Lake 227 was chosen for this study because of the considerable data which were available on the concentrations of CH_4, ΣCO_2 and NH_3-N throughout the year (Rudd and Hamilton 1978; Schindler et al. 1973), and because of the work which had been done on mixing properties of the lake (Hesslein and Quay 1973; Quay et al. 1980). Lake 227 is a small lake (5.0 ha) in the Canadian Shield with a maximum depth of 10

[1]Present address: Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6.

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m and a mean depth of 4 m. It has been part of an eutrophication experiment since 1969. Sodium nitrate and phosphoric acid have been added to the lake during the ice-free season since the spring of 1969. This fertilization has resulted in greatly increased algal standing crop and primary production. The rate of disappearance of oxygen in the deeper waters has increased as well as the deeper water concentrations of CH_4, ΣCO_2, and NH_3-N.

The concentrations of methane, dissolved inorganic carbon, and ammonia are functions of fluxes from the sediments, vertical diffusion, and chemical transformations in the water. Having measured concentration profiles, flux from the sediments and chemical transformation for methane, the magnitude of vertical diffusion can be calculated and then be used in the calculation of flux from the sediments of dissolved inorganic carbon and ammonia.

Hutchinson (1941), in an examination of eddy diffusion and bicarbonate distributions in Linsley Pond, came to the conclusion that the bicarbonate concentration in any stratum of the water column was related to the ratio of the area of sediment to the lake volume at the particular depth interval. He suggested that the mechanism producing this relationship was a high horizontal mixing rate relative to the slow vertical mixing rates which he had calculated. Alsterberg (1927) had suggested a similar mechanism to explain the more rapid disappearance of oxygen at greater depths in lakes. Using the differentiated bathymetric curve, $\partial A/\partial V$, and the bicarbonate distribution with depth, Hutchinson calculated a bicarbonate flux from sediments of $10–15 \times 10^{-3}$ mol·m^{-2}·d^{-1}. This study extends the logic of this approach through the use of numerical methods now easily workable using computers, and takes advantage of the more complete understanding of mixing in lakes which has been developed since Hutchinson's paper.

Imboden and Emerson (1978) used natural radon-222 distributions to estimate vertical and horizontal eddy diffusion coefficients in Greifensee. They found horizontal coefficients exceed vertical coefficients by a factor of 10^3–10^4. They suggest that a one dimensional horizontally well mixed model produces errors of about a factor of two in the estimate of the vertical eddy diffusion coefficient. Their radon and phosphate data show a very important influence of the horizontal mixing and lake basin shape on the shape of concentration versus depth profiles.

Methods

A numerical model was developed to represent the time-dependent distribution (in Lake 227) of sediment-derived dissolved chemical species. The model takes into account the three basic processes which determine this distribution: physical mixing of the water, transport across the sediment–water interface and the thermocline, and chemical transformations in the water. Some conditions have been assumed to simplify the equations describing the physical mixing processes: (1) Net advection is considered to be zero as the lake is a closed system, and on the time scale for which the model was constructed (weeks to months) advection can be lumped into an eddy diffusion term, (2) The system is considered to be well-mixed horizontally in the ice-free season; that is the horizontal eddy diffusion terms are considered to be infinitely large, (3) The vertical eddy diffusion terms are considered to be horizontally uniform. These conditions are supported by experimental work. Measurements of many chemical species including methane, CO_2, and NH_3-N (Stainton et al. 1974), and radon gas (Quay 1977) have shown no significant horizontal gradients in the thermocline or hypolimnion. Experiments on the mixing done with tritium (Quay 1977) show high rates of horizontal eddy diffusion. Even if the horizontal mixing is not rapid, the net vertical transport will not be affected as long as the vertical mixing is of a horizontally uniform rate. That is:

$$(1)\quad \int_x \int_y K_z \frac{\partial c}{\partial z} = K_z \int_x \int_y \frac{\partial c}{\partial z} \text{ for } K_z$$

independent of x and y.

With the above simplifications it is possible to derive an equation for the change in concentrations with time of any dissolved species subject only to vertical eddy diffusion. The total flux through any level of the lake is AF where A is the area and F is the flux per unit area. The flux per unit area can be expressed by Fick's Law as $F = -K(\partial c/\partial z)$ where K is the coefficient of eddy diffusion, c is concentration, and z is depth. The change in concentration with time at any level is just the difference in transport into the level and out of it; that is, the change in total flux with depth. This can be expressed as:

$$(2)\quad \frac{\partial c}{\partial t} = \frac{\partial}{\partial z}\left(-AK\frac{\partial c}{\partial z}\right),\ t = \text{time, which expanded is:}$$

$$(3)\quad \frac{\partial c}{\partial t} = \frac{\partial A}{\partial z} K \frac{dc}{dz} + A\frac{\partial k}{\partial z}\frac{\partial c}{\partial z} + AK\frac{\partial^2 c}{\partial z^2}.$$

Multiplying by $1/A$ we have:

$$(4)\quad \frac{\partial c}{\partial t} = K\left(\frac{\partial^2 c}{\partial z^2} + \frac{1}{A}\frac{\partial A}{\partial z}\frac{\partial c}{\partial z}\right) + \frac{\partial k}{\partial z}\frac{\partial c}{\partial z}.$$

It is worth noting that $1/A\partial z$ in the second part of the first term is equal to the reciprocal of volume of any finite interval at level z. In addition it is important to realize that in contrast to the equation for planar diffusion in an infinite space

$$\left(\frac{\partial c}{\partial t} = K\frac{\partial^2 c}{\partial z^2}\right)$$

a linear gradient of concentration with depth in the lake

$$\left(\frac{\partial^2 c}{\partial z^2} = 0\right)$$

even with K as constant with depth, does not indicate a steady state (no change in concentration with time).

Since the lake is considered to be horizontally homogeneous, the flux at the sediment–water interface can be assumed to be instantaneously distributed at each depth. The addition of an increment in concentration at any level z is the flux times the area of sediment at that level ($F_z A$) divided by volume at that level. Therefore, the change in concentration with time at depth z due to flux from the sediments can be described by:

$$(5)\quad \frac{\partial c}{\partial t} = \frac{F_z \partial A}{A \partial z}$$

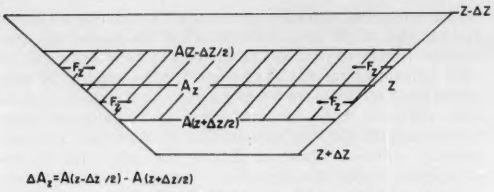

FIG. 1. Generalized layer of the numerical model.

The boundary transport processes controlling F_z can be described as either a concentration-dependent or concentration-independent flux across the boundary. Fick's Law would be expected to apply so that:

$$(6)\quad F_B = -D_B\left(\frac{\partial C}{\partial x}\right)B$$

where F_B is the boundary flux, D_B the boundary diffusion coefficient, and $(\partial C/\partial x)B$ the gradient across it. In the case where D_B is very high (and consequently $(\partial C/\partial x)B$ very low), F_B may no longer be defined by Fick's Law but by the availability of the species at the boundary and independent of the concentration in the bulk. Fluxes at the sediment–water interface have been shown to be governed by both concentration-independent and Fickian processes (Hesslein 1976).

If the sediment–water flux is diffusion-controlled then flux would be inhibited by rising concentration in the water; however, if the production of a chemical species in the sediment is constant with time, its concentration in sediments would increase at the same rate, maintaining the sediment–water gradient. It is assumed for the purposes of this model that the response time of flux from sediments to production of a chemical in the sediment is short relative to the duration of the simulation. This means that the flux from the sediments is equal to the production rate in the sediments and independent of concentration in the water.

If one uses P_z to represent the production-loss term for the chemical transformations in the water the full equation for concentration change at any level z is:

$$(7)\quad \left(\frac{\partial C}{\partial t}\right)_z = \frac{F_z}{A_z}\frac{\partial A}{\partial z} + K_z\left(\frac{\partial^2 C}{\partial z^2} + \frac{\partial A}{A_z \partial z}\frac{\partial C}{\partial z}\right) + \frac{\partial K}{\partial z}\frac{\partial C}{\partial z} + P_z$$

The equation appears in finite difference form in the model as:

$$(8)\quad C_z^{t+\Delta t} = F_z(A_{(z+\Delta z/2)} - A_{(z-\Delta z/2)}) + K_{(z-\Delta z/2)}\left(\frac{C_{(z-\Delta z)} - C_z}{\Delta z}\right)A_{(z-\Delta z/2)} + K_{(z+\Delta z/2)}\left(\frac{C_{z+\Delta z} - C_z}{\Delta z}\right)A_{(z+\Delta z/2)}\frac{\Delta t}{Vz} + P_z t + C_z^t$$

A general picture of one layer is shown in Fig. 1. The values of A and V are taken from the bathymetric curve of the lake (Fig. 2). $C_z^{t=0}$ comes from the concentration versus depth profile at the starting time of the model. The parameters K, C_{sed}, and F are specifiable in each layer. The values of these parameters for various model runs are discussed in the following section. A value of 1 d was chosen for the time step Δt. This proved sufficiently short that no gain in precision was achieved by shortening Δt, yet long enough that running time was not extensive on a small desktop computer. The program was written in BASIC language. Running time was about 40 model d/min real time.

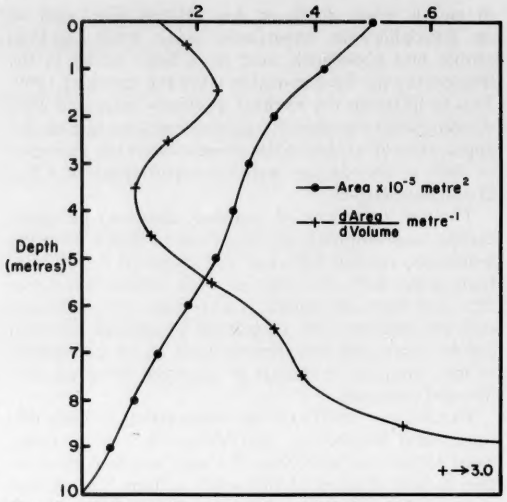

FIG. 2. Bathymetric parameters of Lake 227; area vs. depth and d area/d volume vs. depth.

Results

CONSTANT K_z MODEL — METHANE

The initial approach to the use of the model was to first use methane, which was being extensively studied in Lake 227 (Rudd et al. 1974, 1976; Rudd and Hamilton 1975, 1978) to calibrate the mixing coefficient K_z and then to use the model and that K_z to explore the sediment flux for ΣCO_2 and NH_3. For all cases the flux parameter F_z was assumed to be constant throughout the lake basin. This assumption was made for simplicity, owing to the lack of any direct measurement of flux. Its validity is supported to some extent by the similar nature of the sediments observed in the many cores taken from Lake 227 and in diver observations. Further discussion of the sediments is given by Hesslein (1976). The magnitude of the total sediment flux was estimated by a mass balance calculation of methane appearing in and consumed in the lake over the 168-d period December 18, 1973–June 4, 1974. The calculation was made by subtracting the amount of methane present on December 18, 1973 from that on June 4, 1974 and adding to this the amount of methane oxidized over that period as estimated by Rudd and Hamilton (1975). As Rudd showed that little or no methane evades the methane oxidizers, this should be a complete accounting of the methane produced. A small correction was made for the slight amount of flushing due to gas exchange and outflow during incomplete spring mixing. This amounted to a correction of ~10%. It was assumed that no methane escaped from sediments overlain by oxic water as this would be oxidized in the top layers of sediment (but not measured by Rudd's method). The total amount of methane

thus calculated was therefore divided by a time-weighted area of sediment overlain by oxygen-free water giving a sediment flux average of 1.3×10^{-2} mol·m^{-2}·d^{-1} over the described time period. This number is estimated to be accurate within ~10%.

The mixing coefficient K_z was assumed to be constant vertically and horizontally in the model and with time. This assumption was again made for simplicity and because most studies using temperature methods for calculating K_z showed no large variation of K_z with depth (Hutchinson 1941, 1957; Hesslein and Quay 1973; Li 1973). The temperature method of calculating K_z gives the average over the longest time period and the model was run for about ½ yr. As previously mentioned, methane is conservative in the absence of oxygen in Lake 227 (Rudd et al. 1974, 1976) so the net production-loss term (loss in this case, as production occurs only in the sediment) becomes a boundary condition. That boundary is the level of 0.1 mg/L oxygen concentration (Rudd et al. 1974, 1976). It is a floating boundary in the model as the 0.1 mg/L oxygen level varies in position throughout the season. From Rudd's work (1975) it is clear that little or no methane escapes the oxidizers if more than 0.1 mg/L oxygen is present and that the methane concentration decreases very rapidly in this zone of oxidation, which is usually only 0.25 m thick. This gives rise to the simple observation that if oxygen is present at greater than 0.1 mg/L the methane concentration is near zero. This condition holds except at spring and fall overturn when oxygen and methane-rich waters are mixed more rapidly than the oxidizers are able to work. The analog for consumption of methane by oxidation is thus written: if oxygen exceeds 0.1 mg/L in any layer the methane concentration in that layer is defined as zero. The functioning of this analog then depends on oxygen data (Table 1) for the lake over the time period which the model is run. Real data for oxidation from Rudd and Hamilton (1978) could be substituted for the oxygen analog, but there would be no gain in accuracy and oxidation data are far more scarce in other lake studies than oxygen concentration.

Concentration profiles of methane were generated by the model for dates on which measured methane concentration profiles were available (Rudd and Hamilton 1975) to simplify comparison. Because the purpose of the methane modeling was to calibrate the model for vertical mixing, a range of values for K_z was tried. A least-squares comparison of the model generated versus the measured concentration profiles was carried out for all dates for which methane data were available in order to arrive at a best fit for K_z. The form of this analysis is $\chi^2 = \Sigma(C_{\text{mod}} - C_{\text{meas}})^2/C_{\text{mod}}$. This function when plotted against K_z gives a parabolic shape, the minimum of which is the best agreement between the two sets of numbers and the K_z at that point the best fitting K_z.

Best fit K_z values (in cm^2/s) for model runs starting on December 18, 1973 are, respectively, for June 4, July 2, July 30, August 28, 2.8×10^{-3}, 3.3×10^{-3}, 3.1×10^{-3}, 2.25×10^{-3}. Actual comparison of the model-generated and measured data shows just how well the model is able to simulate the lake system (Fig. 3).

TABLE 1. Oxygen in Lake 227 — 1974 (mg/L). Data from ELA group, Freshwater Institute, Winnipeg, Manitoba. (Zero concentration represented by "—".)

Depth (metres)	Day number						
	352/73	22	92	126	141	155	169
3	2.05	0.15	—	—	6.09	10.62	10.62
4	1.58	0.02	—	—	2.89	2.58	3.70
5	0.40	0.01	—	—	0.25	0.12	—
6	0.12	—	—	—	—	—	—
7	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—
	183	197	211	225	239	253	267
3	10.26	10.40	11.94	12.47	8.42	9.00	10.20
4	0.40	4.61	1.57	0.98	1.20	4.35	9.61
5	—	—	—	0.06	—	0.15	0.03
6	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—

In viewing the model results it is worth keeping in mind a few general characteristics of this sediment-flux water-mixing system. An increase in the vertical mixing coefficient K_z serves to decrease the gradient in the system and to decrease the mass of methane, in this case by supplying it to the oxidizers (or specifically the oxidizer analog) at a greater rate. A decrease in the flux has the same effect, of course, decreasing the mass in the system but also decreasing the gradient since the function $\partial A/Adz$ (from the flux equation) increases with increasing depth (Fig. 2, dA/dV).

The values of K_z generated by the methane model (2.8–3.3×10^{-3} cm^2·s^{-1}) agree very well with other determinations in Lake 227. Hesslein and Quay (1973) published values of K_z for the region from 3 to 8 m depth calculated from temperature by the method of McEwen (1929). These values range from 3.3 to 3.8×10^{-3} cm^2·s^{-1}. We also presented results from radon and dye experiments in limnocorrals which gave K_z for the 2.5–4 m zone to be, respectively, 0.5×10^{-3} cm^2·s^{-1} and 1.2×10^{-3} cm^2·s^{-1}. Since that publication Quay et al. (1979 unpublished data) have redetermined the value of K_z for a number of years by a finite difference method using heat transport. The values for K_z from these determinations range from 1.5×10^{-3} to 3.5×10^{-3} cm^2·s^{-1}. Correcting for the molecular diffusion of heat, this gives 0.3×10^{-3} to 2.3×10^{-3} cm^2·s^{-1}. Most recently (Quay et al. 1980) the vertical eddy diffusion coefficients in the metalim-

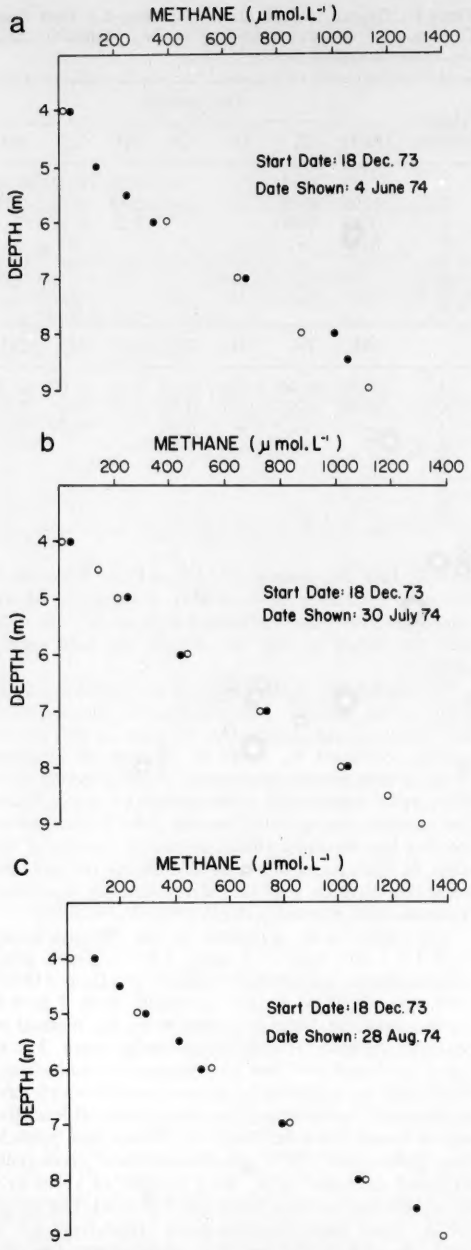

FIG. 3. Comparisons of measured (●) and model generated (○) methane concentrations for three dates in 1974, Lake 227. Start date for the model and date of comparison are given.

nion and hypolimnion have been determined by a tracer experiment in the whole lake using tritiated water. This experiment gives values of K_z at 6–9 m depth for a period of 5 wk in late summer by two methods of analysis of $1.7 \pm 0.2 \times 10^{-3}$ cm$^2 \cdot$s^{-1} and $2.4 \pm 0.7 \times 10^{-3}$ cm$^2 \cdot$s^{-1}. All these values are generally compatible with those found by the methane lake model. The metalimnion value for K_z from the tritiated water experiment was 0.05×10^{-3} cm$^2 \cdot$s^{-1}. This is much lower than the values arrived at by the methane model. This indicates a variation of K_z versus depth as discussed by Quay et al. (1980). This problem is looked at in greater detail later in this paper.

After establishing a value for K_z based on the best fit of modeled methane distribution against measured values, the fluxes and resulting distributions of ΣCO_2 and NH_3-N were modeled using the K_z determined in the methane simulation. The best fit fluxes were determined in the same manner as K_z in the methane model. Different boundary analogs were required for both ΣCO_2 and NH_3-N. Since ΣCO_2 is not consumed in the presence of oxygen, the oxygen concentration could not be used as an effective "top" to the model. The oxygen concentration after the disappearance of ice cover represents the effective penetration, by mixing, of surface waters. This mixing also allows ΣCO_2 to equilibrate with the overlying water. Empirically this results in a ΣCO_2 concentration of ~270 mol/L in the presence of significant amounts of oxygen below the mixed layer. The analog was thus written: if the oxygen concentration is greater than 5 mg/L and the lake is ice free, $\Sigma CO_2 = 270$ μmol/L. Similarly for nitrogen the analog was written: if the concentration is greater than 6 mg/L and the lake is ice free, NH_3-N = 3 μmol/L and the NH_3-N flux from sediments is zero if the oxygen concentration is greater than 0.1 mg/L.

The best fit for ΣCO_2 flux with $K_z = 3.1 \times 10^{-3}$ cm$^2 \cdot$s^{-1} is 7.5×10^{-3} mol$\cdot$m$^{-2} \cdot$d^{-1} (Fig. 4). This

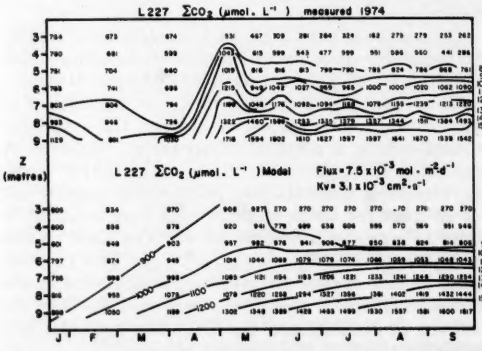

FIG. 4. Isopleth plots of measured and model generated ΣCO_2 concentrations 1974, Lake 227. (Measured values from J. E. Prokopowich unpublished data).

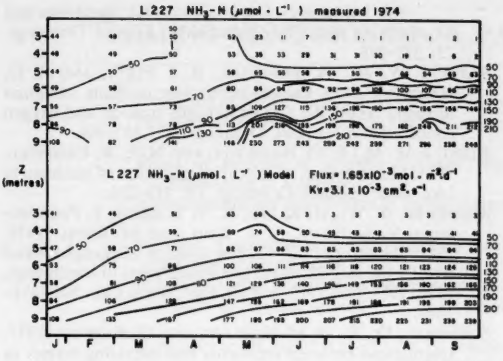

FIG. 5. Isopleth plots of measured and model generated NH_3-N concentrations 1974, Lake 227. (Measured values from J. E. Prokopowich unpublished data).

represents the minimum value of χ^2 averaged over the ice-free period of June 4 to September 10. The measured concentrations show a rapid rise after the disappearance of ice cover. This could be due to incomplete horizontal mixing under winter ice cover. Samples were taken at the center of the lake and could underestimate the mean concentration under ice. As pointed out in the earlier section on the theory of the model, this will not affect the resultant ice-free profiles as long as K_z is horizontally uniform.

The best fit for NH_3-N flux with $K_z = 3.1 \times 10^{-3}$ $cm^2 \cdot s^{-1}$ is 1.65×10^{-3} $mol \cdot m^{-2} \cdot d^{-1}$. Isopleth plots for comparison of model versus measured concentrations (Fig. 5) show that as with ΣCO_2 there is a rapid rise in concentration of measured NH_3-N in the spring possibly due to increased horizontal mixing.

Discussion

Taking the best estimates of the fluxes of CH_4, ΣCO_2, and NH_3-N from the sediments to be, respectively, 13×10^{-3} $mol \cdot m^{-2} \cdot d^{-1}$, 7.5×10^{-3} $mol \cdot m^{-2} \cdot d^{-1}$, and 1.65×10^{-3} $mol \cdot m^{-2} \cdot d^{-1}$ the molar carbon:nitrogen ratio $((CH_4 + \Sigma CO_2)/NH_3\text{-N})$ is 12.4. This can be compared with a ratio of 13.4 found by Brunskill et al. (1971) in a grab sample of surface (0–20 cm) sediments of Lake 227. Hesslein (1980) found a ratio of 14.9 for a dried sediment in a core from 1.5 m in Lake 227. Carbon-to-nitrogen ratios in the pore waters of Lake 227 range from 12 to 15 (Hesslein 1976). Schindler et al. (1973) have reported a molar C:N ratio in suspended particulate material in the range of 7–10 for the summer epilimnion.

The molar ratio of CH_4 to ΣCO_2 from the flux estimates is 1.73. This is in the range of the ratios summarized by Robertson and Chynoweth (1977) 1.5–2.2. This range includes values determined by fermentation of algae in lakes, degradation of domestic sewage and in vitro fermentation of lake sediments. As mentioned earlier the conditions for the model do not allow any CH_4 or NH_3-N flux from the sediments if those sediments are overlain by oxic water. This is not true for ΣCO_2. The ΣCO_2 flux in the model is held constant independent of oxygen concentration. It might seem logical that the total carbon flux from the sediment water interface would remain constant. The model would then use the sum of CH_4 flux + ΣCO_2 flux for ΣCO_2 if the sediments were overlain by oxic water and ΣCO_2 alone under anoxic conditions. The best fit values for the model using these assumptions give very poor agreement with the measured data. Possibly a greater percentage of decomposed carbon would be fixed as cell carbon by bacteria in the presence of oxygen. The problem of decomposition products in zones where oxygen concentrations are variable appears to be worthy of further microbiological studies.

A more sophisticated approach to the mixing parameter K_z will be possible in future models of this sort. Quay (1977) and Quay et al. (1980) have presented a solid empirical relationship between the density gradient (Brung-Vaisala frequency) and the vertical eddy diffusion coefficient in lakes. If temperature data over time were available for a lake, a two-dimensional array of K_z versus depth and time could be constructed to replace the constant K_z used in the models presented here. Unfortunately, this relationship between stratification and eddy diffusion has only been demonstrated for ice-free conditions. Its application under ice is uncertain.

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